Malaria Chemoprevention for the Post-Discharge Management of Paediatric Severe Anaemia in Malaria Endemic Areas of Africa

Thesis submitted in accordance with the requirements of the

Liverpool School of Tropical Medicine

for the degree of Doctor in Philosophy by:

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I would like to thank my family and friends for their encouragement and support.
I cannot find the words to express my gratitude to my dear wife, Millicent and our children; Kibet, Jepchumba and Kigen for their patience and support throughout this journey.
This thesis is dedicated to the persons who have nurtured me throughout my life, my parents, Mr & Mrs Albert C. Kwambai. Most highly, I thank God for the good health and the ability to pursue this course.

Declaration

I hereby declare that this PhD thesis is a presentation of my original research work. Material contained herein has not been previously published, accepted or presented for the award of any University degree. Wherever contributions of others are involved, every effort has been made to indicate this clearly, with due acknowledgement to the relevant sections made in the thesis.

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List of abbreviations

ACT	Artemisinin-based Combination Therapy
AE	Adverse Event
AL	Artemether-Lumefantrine
AQ	Amodiaquine
ART	Antiretroviral Therapy
AS	Artesunate
bpm	Breaths Per minute
CDC	Centers for Disease Control and Prevention
CGHR	Centre for Global Health Research
CI	Confidence Interval
CLQTS	Congenital Long QT Syndrome
CQ	Chloroquine
D+L	DerSimonian and Laird
DBS	Dry Blood Spot
dhfr	Dihydrofolate Reductase
dhps	Dihydropteroate Synthetase
DP	Dihydroartemisinin-Piperaquine
DSMB	Data Safety and Monitoring Board
ECG	Electrocardiogram
EDCTP	European and Developing Countries Clinical Trials Partnership
FUP	Follow-Up Period
G6PD	Glucose-6-Phosphate Dehydrogenase
GCP	Good Clinical Practice
GLOBVAC	Global Health and Vaccination Research
GMP	Good Manufacturing Practice
HAZ	Height for Age Z-Score
Hb	Haemoglobin
HDSS	Health and Demographic Surveillance System
HIV	Human Immunodeficiency Virus

HR	Hazard Ratio
HRP-2	Histidine-Rich Protein-2
ICH	International Conference on Harmonisation
IMCI	Integrated Management of Childhood Illnesses
iNTS	Invasive Non-typhoidal Salmonella
IPD	In-Patient Department
IPT	Intermittent Preventive Therapy
IPTc	Intermittent Preventive Therapy in Children
IPTi	Intermittent Preventive Therapy in Infants
IPTp	Intermittent Preventive Therapy in Pregnancy
IPTpd	Intermittent Preventive Treatment Post-Discharge
IQR	Interquartile Range
IR	Incidence Rate
IRR	Incidence Rate Ratio
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Bed Nets
ITT	Intention-to-Treat
JOOTRH	Jaramogi Oginga Odinga Teaching and Referral Hospital
KEMRI	Kenya Medical Research Institute
LBW	Low Birthweight
LLINs	Long-Lasting Insecticide Treated Nets
LSTM	Liverpool School of Tropical Medicine
MD	Mean Difference
MDGs	Millennium Development Goals
MedDRA	Medical Dictionary for Regulatory Activities
MHOR	Mantel-Haenszel Odds Ratio
MIP	Minimum Parasiticidal Concentrations
mm Hg	Millimetres of Mercury
MQ	Mefloquine
MR	Mortality Rate

MRR	Mortality Rate Ratio
ms	Milliseconds
MUAC	Mid-Upper-arm-Circumference
NOS	Newcastle Ottawa Scale
OPD	Outpatient Department
PD	Post-Discharge
PE	Protective Efficacy
PEM	Protein-Energy Malnutrition
PMC	Post-Discharge Malaria Chemoprevention
PP	Per-protocol
PQ	Piperaquine
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PWP-GT	Prentice Williams Peterson–Gap Time
PWP-TT	Prentice Williams Peterson-Total Time
PY	Person-Years
RBC	Red Blood Cells
RCPF	Red Blood Cell Production Failure
RCT	Randomised Controlled Trial
RDT	Rapid Diagnostic Test
RR	Rate Ratio
RR	Relative Risk
RRR	Relative Rate Reduction
RSV	Respiratory Syncytial Virus
SA	Severe Anaemia
SAE	Severe Adverse Event
SAM	Severe Acute Malnutrition
SD	Standard Deviation
SDG	Sustainable Development Goals
SES	Socioeconomic Status
SMA	Severe Malarial Anaemia

SMC	Seasonal Malaria Chemoprevention
SOMREC	School of Medicine Research and Ethics Committee
SP	Sulphadoxine-Pyrimethamine
SPO_2	Peripheral Capillary Oxygen Saturation
SSA	sub-Saharan Africa
TB	Tuberculosis
TS	Trimethoprim-Sulfamethoxazole
TSC	Trial Steering Committee
UN	United Nations
VL	Visceral Leishmaniasis
WAZ	Weight for Age Z-Score
WHO	World Health Organization
WHZ	Weight for Height Z-Score

Thesis Abstract

Children hospitalised with severe anaemia in malaria-endemic areas are at high risk of readmission or death within six months post-discharge. No strategy specifically addresses this post-discharge period. This thesis aims to provide evidence of the burden of post-discharge mortality and morbidity among children less than five years of age who are admitted with all-cause severe anaemia and other syndromes and living in malaria-endemic areas of Africa. We also report the results of a malaria chemoprevention trial for the post-discharge management of severe anaemia.

In the first study, we conducted a retrospective cohort analysis using data collected between 2008 and 2013 from continuous paediatric in-hospital surveillance and population-based surveillance in western Kenya. During this period, 3,639 hospital admissions involving 4,423 different diagnoses were recorded. Overall, in-hospital mortality was 2.8% and the post-discharge mortality by three, six and twelve months among the 3,538 survivors was 7.6%, 9.4%, and 12.4%, respectively. Admissions with severe acute malnutrition and severe anaemia were associated with the highest post-discharge mortality in the first six months. Severe anaemia was associated with a significantly higher odds of six-month post-discharge mortality than in-hospital mortality (Mantel-Haenszel odds ratio [MHOR]=2.02, 95% CI 1.19-4.05, p=0.011).

In the second study, we conducted a systematic review and meta-analysis to determine the pooled risks of morbidity and mortality in the post-discharge period among children admitted with severe anaemia versus other syndromes in malaria-endemic areas in Africa. Children admitted with severe anaemia were found to have higher odds of dying within six months post-discharge than during the in-hospital stay (N=4, MHOR=1.44, 95% CI 1.07-1.92, p<0.0157, I^2=0.8%) and they were twice as likely to die by 6 months post-discharge compared with children admitted without severe anaemia (N=4, Relative Risk [RR]=2.80, 95% CI 1.61-4.86, p<0.0001, I^2=73.1%). Severe acute malnutrition was also associated with increased post-discharge mortality compared to children admitted without severe anaemia or malnutrition (N=2, RR=4.27, 95% CI 2.42-7.55, p<0.0001, I^2=76.8). The risk of readmission was also higher among children admitted with severe anaemia compared to other syndromes (RR=3.05, 95% CI 1.12-8.35, p<0.001, I^2=0.0%).

The third study was a randomised placebo-controlled trial in nine hospitals in Kenya and Uganda to determine if three months of post-discharge malaria chemoprevention (PMC) with monthly 3-day treatment courses of dihydroartemisinin-piperaquine (DP) reduced the rate of all-cause

readmissions and deaths by six months post-discharge (primary outcome). Children aged <5 years with admission haemoglobin of <5g/dL were eligible. A 3-day course with artemether-lumefantrine was given to all children at discharge. Children were then randomised to receive DP or placebo-DP at two, six, and ten weeks post-discharge and followed until week 26 inclusive. There were 184 primary outcome events in the PMC arm and 316 in the placebo arm (HR=0.65, 95% CI 0.54-0.78, p<0.001). The HR was 0.30 (95% CI 0.22-0.42, p<0.001) during the PMC-intervention period (2-14 weeks) and 1.13 (95% CI 0.87-1.47, p=0.35) during the post-intervention period (15-26 weeks), p<0.001).

This thesis shows that children admitted with severe anaemia and other acute conditions are at a high risk of post-discharge mortality and morbidity. Admission with all-cause severe anaemia in this setting is associated with a doubling of the risk of post-discharge mortality compared to other acute conditions. Malaria chemoprevention with three monthly courses of DP is a promising tool for post-discharge management of children recently admitted with severe anaemia in malaria-endemic areas.

Statement of involvement of the PhD candidate in the PMC trial

The PMC trial was conducted in hospitals situated in areas with moderate to intense malaria transmission around Lake Victoria in Kenya and Uganda between May 2016 to November 2018. This was one of the five major activities of the post-discharge malaria chemoprevention consortium funded by the Global Health and Vaccine Research program (GLOBVAC) through the University of Bergen, Norway. The consortium had five major objectives including the PMC confirmatory safety and efficacy trial; a delivery mechanisms trial and health services research in Malawi to determine the uptake, effectiveness, acceptability and feasibility of PMC delivery mechanisms; PMC intervention impact analysis involving systematic reviews, meta-analyses and mathematical modelling to determine the public health impact of PMC; economic evaluation of the intervention and a policy liaison task force with the World Health Organization (WHO).

This thesis includes a report of the findings of the confirmatory safety and efficacy trial conducted in Kenya and Uganda as well as the findings of the systematic review and meta-analysis of the post-discharge burden of severe anaemia in malaria-endemic areas of Africa.

The study was conceived by Prof Feiko ter Kuile and Prof Kamija Phiri. The original protocol was drafted prior to the recruitment of the PhD candidate Dr Titus Kwambai, by Prof Feiko ter Kuile, Prof Kamija Phiri, Prof Chandy C John, Dr Richard Idro and Dr Robert Opoka. Additionally, Dr Titus Kwambai, Dr Aggrey Dhabangi, Dr Meghna Desai, Dr Simon Kariuki, Prof Michael Boele van Hensbroek and Prof Bjarne Robberstad helped to further develop the study design in a protocol workshop while Prof Duolao Wang provided statistical expertise. Dr Titus Kwambai, Dr Aggrey Dhabangi and Prof Feiko ter Kuile drafted the protocol amendments.

Dr Titus Kwambai and Dr Aggrey Dhabangi were responsible for developing the study data collection tools, standard operating procedures, recruitment and training of study personnel and set up of field sites under the local supervision of Professor Feiko ter Kuile and Dr Simon Kariuki in Kenya and Dr Richard Indo and Dr Robert Opoka in Uganda. In Kenya, Mr Nickline Ashitiba supervised the daily activities of the field staff under the supervision of Dr Titus Kwambai. Mr Kephas Otieno and Telesphorus Odawo coordinated the laboratory work. Prof Feiko ter Kuile had the overall responsibility for the trial. Dr Titus Kwambai was directly responsible for drafting the protocol amendments, budgeting and procurement of study supplies; staff training and appraisal, data collection and documentation as per good clinical practice guidelines; coordination of laboratory, field activities and interaction with the Ministry of Health and study hospitals in Kenya;

coordination of trial monitoring activities; safety monitoring and correspondence with ethics review committees of the Kenya Medical Research Institute (KEMRI), and the LSTM; a compilation of annual reports to KEMRI, LSTM and CDC.

Dr Titus Kwambai and Mr Eric Onyango were responsible for data cleaning and reporting. Drafting of the data analysis plan and data analysis were conducted by the Dr Titus Kwambai and Ms Vicky Watson in consultation with Prof Feiko ter Kuile, Prof Duolao Wang and Dr Tao Chen.

Dr Titus Kwambai did the data management and conducted the data analysis, for the retrospective cohort study (Chapter 3) and the systematic review and meta-analysis (Chapter 4). Ms Victoria Watson provided statistical support for Chapter 6, and Dr Sarah Nevitt provided statistical support and advice for Chapters 3 and 4.

This thesis was prepared and written by the PhD candidate with guidance and support from the PhD supervisors.

Chapter 1: Preface

General Introduction

Severe anaemia due to severe malaria or other acute or chronic conditions is a leading cause of hospital admissions and mortality in the paediatrics population in malaria-endemic areas of Africa. Children under five years of age admitted with severe anaemia have an equal or higher risk of death or readmission for several months after discharge compared to the acute initial in-hospital period.[1] This is a neglected high-risk period. Earlier studies in high malaria transmission areas of Kenya and Malawi showed that malaria in the post-discharge period is an important contributor responsible for slow haematological recovery, rebound severe anaemia and morbidity in this high-risk population.[2] Currently, there is no strategy that addresses this potentially preventable component of the burden of severe anaemia. A proactive approach using several months of post-discharge malaria chemoprevention could offer substantial public health gains.

This thesis is designed to provide further evidence of the burden of severe anaemia in the post-discharge period in children living in malaria-endemic areas of Africa and reports the results of a multi-site, double-blind placebo-controlled trial of three months of malaria chemoprevention to reduce all-cause mortality and readmission in the post-discharge management of young children with severe anaemia in malaria-endemic areas in Africa.

Thesis Outline

- **Chapter 1:** Preface
- **Chapter 2:** The Literature review.
- **Chapter 3:** Describes the findings of a retrospective cohort analysis of historical data from a Health and Demographic Surveillance Site (HDSS) in western Kenya on the relationship between hospital admission with severe anaemia or other syndromes and the post-discharge risk of mortality in children under five years of age.
- **Chapter 4:** Presents the findings of a systematic review and meta-analysis of post-discharge risks of morbidity and mortality in children admitted with severe anaemia and other syndromes in malaria-endemic settings in Africa.

- **Chapter 5:** Presents the study protocol for the randomised controlled trial on malaria chemoprevention with monthly dihydroartemisinin-piperaquine for the post-discharge management of severe anaemia in children aged less than five years in Uganda and Kenya.
- **Chapter 6:** Describes the findings of the randomised controlled trial (chapter 5) and discusses the results.
- **Chapter 7:** Provides the summary and discussions and gives recommendations based on the current body of knowledge.

Chapter 2: Literature Review

The burden of malaria in children

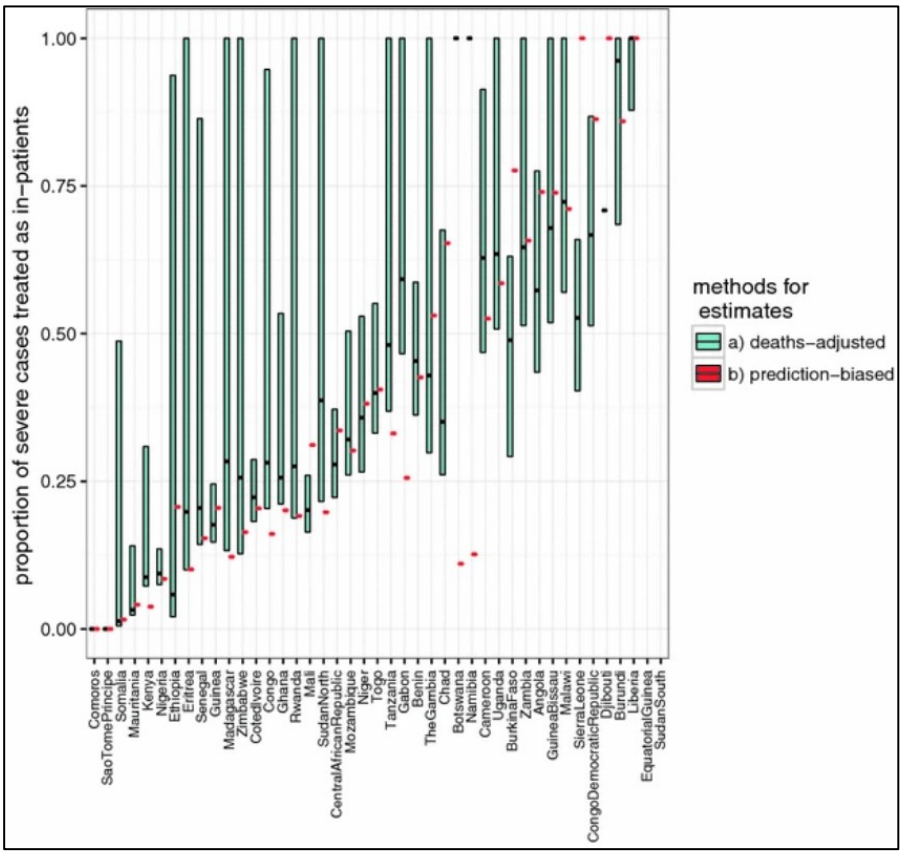
Remarkable progress in reducing under-five mortality rates globally was made between 1990 and 2015 under the United Nations (UN) Millennium Development Goals (MDGs) agenda where under-five mortality fell from 90.6 to 42.5 deaths per 1000 live births.[3] Globally 5.5 million children died with sub-Saharan Africa contributing 2.8 million deaths in 2017.[4] Despite being a largely preventable and treatable disease, malaria accounted for 219 million new cases resulting in 435,000 deaths globally with sub-Saharan Africa disproportionately contributing 93% of these deaths of whom 266,000 were children under five years of age constituting 61% of global malaria deaths.[5] Malaria is a major underlying condition and a significant contributor to severe anaemia, malnutrition and low birth weight in sub-Saharan Africa.[6,7]

Severe malaria has been shown to lead to physical and neurocognitive impairments including spastic motor weakness, hearing deficits, loss of speech, blindness, epilepsy and behavioural problems in children which may manifest in the immediate post-discharge period or later in life as children are faced with more complex cognitive and linguistic demands, socially and educationally.[8,9] Treatment of malaria has substantial direct and indirect costs to households and the whole economy. In Kenya, it costs up to US$ 38.95 to treat a child under five years of age with severe malaria anaemia,[10] in Malawi this was US$ 17.48 in 2012,[11] while the out-patient care in Ethiopia was estimated at US$ 5.06 per visit for persons with a mean age of 16 years in 2015.[12] Malaria is a leading cause of school absenteeism[13] and has been estimated to reduce economic growth in African countries by 1.3%[14] and contributes substantially to the vicious cycle of disease and poverty.[15]

Successful strategies for the control of malaria, e.g. Insecticide-treated bed nets (ITN), Indoor residual spraying (IRS) and treatment with artemisinin-based combination therapy (ACT) have markedly contributed to the decline in the prevalence of malaria cases and deaths and the proportion of fevers due to malaria is decreasing in many malaria-endemic countries in Africa.[5,16] Malaria deaths in the general population in sub-Saharan Africa have decreased by 57% since 2000 (from 12.5 to 5.4 per 10,000 population) with deaths in children under five years reducing from 25 to less than 1 per 10,000 children[17] however, the decline has stagnated since 2015.[5] Estimation of malaria attributable deaths using severe malaria case rates and in-hospital data from malaria-

endemic countries in Africa varies widely. This is partly reflecting the fact that in many countries malaria patients who are admitted do not always meet the strict WHO criteria for severe malaria (i.e. malaria with signs of severe illness and/or evidence of vital organ dysfunction[18]) while in other settings many severe malaria cases do not make it to the hospital and die at home (Figure 1).[19] Therefore, low in-patient malaria case-fatality rates reported in countries where uncomplicated malaria cases form a high proportion of hospitalised children underestimate the proportion of malaria-related deaths and poor-quality in-patient care or delay in accessing care may overestimate the malaria case-fatality rates.

Figure 1: Estimates of the proportion of severe cases receiving in-patient care



Adapted from Componovo et al.[19]
Country estimates of the proportion of severe cases receiving in-patient care by the method of estimation. *Colour* indicates method with the prediction biased estimate in *orange* and the deaths-adjusted estimate in *green*. For the deaths adjusted estimate, the bar indicates the min and max range, and *black* the mean.

The burden of anaemia in children

Anaemia affects about a third of the world's population with 89% of the cases coming from developing countries, the majority of whom are women of reproductive age and pre-school

children with severe anaemia, defined as haemoglobin concentration (Hb) <5g/dL,[20] constituting 3.9% of the overall burden.[21] The global estimate of the prevalence of anaemia in children under five years of age in 2011 was 42.6% and was higher than that for non-pregnant (29.0%) and pregnant women (38.2%).[22] It is, however, acknowledged that these figures may be slightly exaggerated for children under five years of age because the authors defined any anaemia as Hb <12.0g/dL and not <11.0g/dL as defined by WHO.[23] Regional variations in the prevalence of severe anaemia is apparent with malaria-endemic Africa suffering the most. However, the prevalence reduced from 1.1-9.7% in 1995 to 0.9-4.9% in 2011.[24] The high prevalence of anaemia in the African region is not surprising as it mirrors the high prevalence of factors contributing to severe anaemia such as nutritional deficiencies, malaria, etc. and the changes over time possibly reflect the improvements in control measures for diseases such as malaria.

Severe anaemia has been shown to have significant adverse health consequences and negative impacts on social and economic development.[25,26] Severe anaemia in the acute stage causes fatigue, weakness, difficulty in concentrating and poor productivity due to impaired tissue oxygenation and in the long term it has been shown to have impairment in neurocognitive ability,[27] motor development and heart failure.[28] Iron deficiency also leads to impairment of the immune system predisposing to infections.[29]

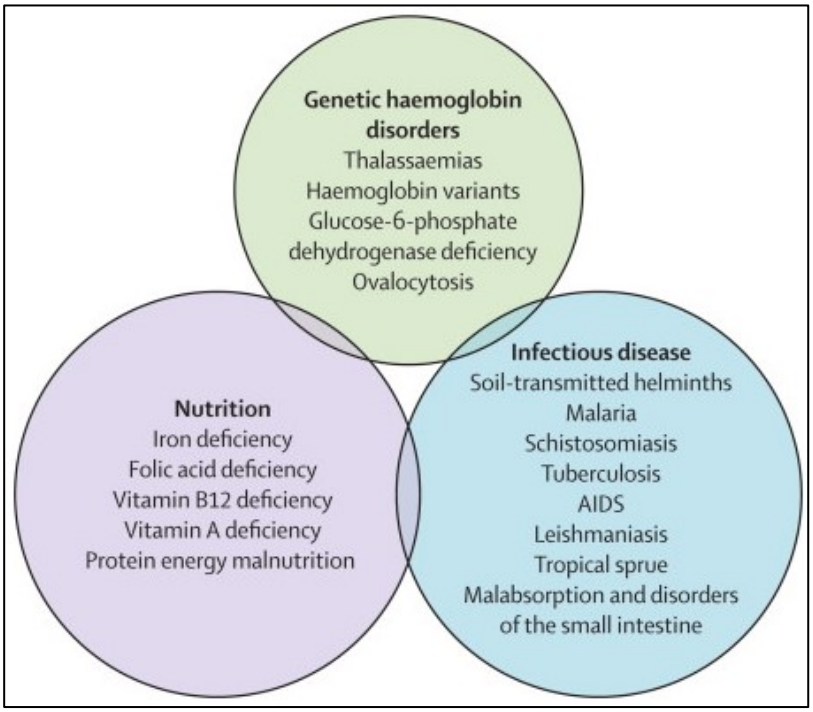
Studies in different epidemiological settings in malaria-endemic Africa show that overall 12 to 29% of hospitalised children are severely anaemic[30-33] as well as about a third of those hospitalised with fever.[34] Mortality during the in-hospital period ranges from 4 to 13% in different epidemiological settings[35-38] and post-discharge mortality is also substantial. Most cases of mild-moderate anaemia progress to severe anaemia if the underlying conditions are not treated in time. Because of ignorance about severe anaemia, community and cultural perceptions, late presentation to health care centres and use of traditional medicines at home contribute to the high prevalence rates of severe anaemia in malaria-endemic areas in Africa.[39] Delay in seeking care, self-medication and seeking care from drug shops also contribute to high mortality and morbidity.[40]

Aetiology and pathophysiology of anaemia in children

The amount of circulating red blood cell mass and functionality of haemoglobin determines the efficiency to which gaseous exchange occurs in the body. Any condition, occurring singly or in combination, that reduces the mass of circulating red blood cells (RBC) or causes a shortage of functional haemoglobin causes anaemia. The aetiology of anaemia can be broadly classified into

decreased erythrocyte production (ineffective erythropoiesis) as a result of the impaired proliferation of red-cell precursors or ineffective maturation of erythrocytes; or increased loss of erythrocytes through increased destruction (haemolysis) or blood loss; or both. The contribution of these factors to severe anaemia is determined by the complex interrelationship between nutrition, infectious diseases and individual genetic predisposition (Figure 2).41 A more detailed review of these individual factors are given below

Figure 2: Causes of anaemia in countries with low or middle incomes



Adapted from: Balarajan et al.[41]

Nutritional factors

Globally, Iron deficiency accounts for approximately 50% of the cases of anaemia, though this varies in different geographical settings and population groups.[23,24] As a result, for a long time, universal iron supplementation was recommended to children living in areas with a high prevalence of iron deficiency. Iron is a crucial component for erythropoiesis and the human immune system, as well as a critical element for the survival of many pathogens. Finding an equilibrium between these factors and the use of iron for the management of anaemia is a current dilemma.[42] Iron deficiency is known to create a hostile environment for bacterial growth, and thus Iron supplementation studies in areas with a high prevalence of bacterial infections reported an increase in morbidity and

mortality.[43,44] However, a study in Malawi in areas with perennial or seasonal malaria transmission found that vitamin A deficiency and not iron deficiency was the most prevalent aetiological factor associated with severe anaemia[45] and vitamin A deficiency was found in 92.3% of participants with severe anaemia. In a structural equation model, the authors showed that the presence of malaria infection and bacteraemia potentiate the association between vitamin A deficiency and severe anaemia. Iron deficiency was shown to be protective for severe anaemia, likely due to its inverse relationship with bacteraemia (Figure 2). Because of the complex interrelationship between iron deficiency, bacteraemia and malaria, WHO now recommends daily iron supplementation for children living in malaria-endemic areas with a high prevalence of anaemia provided malaria prevention is guaranteed.[46]

A Malawian study in 2008 in areas with seasonal or intense year-round malaria transmission showed that about 30% of severely anaemia children have vitamin B_{12} deficiency (OR=4.3, 1.9-9.9 compared to children without severe anaemia).[1] The main cause of vitamin B12 deficiency in this setting is an inadequate nutritional intake of the main sources of vitamin B12 (animal proteins). In Malawi, severely anaemic children ate fewer meals with meat compared to those without severe anaemia (1.9 vs 2.7 per month, P = 0.02).[1] Other causes of vitamin B12 deficiency common in this setting include impaired gastric absorption, e.g. due to pernicious anaemia, impaired intestinal absorption (e.g. due to parasitic infestations: giardiasis, bacteraemia etc.) and increased requirements due to HIV infections or haemolysis (e.g. due to malaria).[47]

The prevalence of folic acid deficiency in children in malaria-endemic areas is low[1] and its contribution to severe anaemia is unclear.[48] A 1995 trial conducted in The Gambia among children with malaria-associated anaemia did not find any improvements in Hb concentrations following folate supplementation.[49] A more recent multivitamin and multimineral supplementation study in Uganda among children with severe anaemia did not show any significant beneficial effects in reducing the burden of severe anaemia in children.[50] The current clinical guidelines for the management of malaria-associated anaemia in Kenya and Uganda which recommends routine iron and folic acid supplementation may need to be reviewed.[51,52]

Infectious diseases

Malaria

Malaria is recognised as the principal cause of anaemia in malaria-endemic areas of Africa. As a result, anaemia has been recommended by WHO as an additional indicator to monitor the performance of malaria control interventions such as ITNs and IRS in areas with stable malaria transmission.[41] (see the section below on "The burden of severe malaria anaemia" for a detailed discussion on malaria anaemia).

Soil-transmitted helminths

The most prevalent soil-transmitted helminths in sub-Saharan Africa among school-aged children are hookworms, Ascaris lumbricoides, and Trichuris trichiura at 16·5%, 6·6%, and 4·4% (between 2000 and 2013) respectively. This prevalence has reduced from as high as 52% to 74% as reported in surveys conducted before 2000.[53] Most hookworm infections are in children less than two years of age, with Ancylostoma duodenale being more prevalent than Necator americanus; 80.6% and 8.3% respectively, and 11.1% of the children have mixed infections. In this setting, 15.9% of severe anaemia cases are attributable to hookworm infections.[1] Hookworms cause iron deficiency anaemia with the severity being dependent on the intensity of infection and species (Ancylostoma duodenale is more invasive than Necator americanus).[54] The ecological conditions in sub-Saharan Africa favour larval development with most infections occurring in areas of poverty, where poor water, sanitation, and infrastructure result in endemicity. Treatment with anthelminths and iron supplementation is the primary management strategies.[55]

Schistosomiasis

More than 90% of the global burden of schistosomiasis occurs in sub-Saharan Africa.[56] About three-quarters of the sub-Saharan African population live near water bodies and irrigation projects contaminated with intermediate snail hosts. Most of the burden is in Nigeria, Ghana and Tanzania.[57] In 2011 in Zimbabwe, the prevalence of *Schistosoma* haematobium in 1-5-year-old children was 21%.[58] In 2014 in Uganda, the prevalence of *Schistosoma mansoni* was 39.3% in the shores of Lake Victoria,[59] while in western Kenya, the prevalence was 14% in the 1-year-olds and more than 90% in children > 10 years of age.[60] *Schistosoma haematobium* and *Schistosoma mansoni* cause the highest burden of disease.[56] The mechanism of anaemia includes chronic blood loss leading to iron deficiency anaemia, splenic sequestration and haemolysis due to splenic hypertrophy, autoimmune haemolysis and prolonged pro-inflammatory cytokine-induced anaemia

of chronic disease.[61] The primary drug for the management of schistosomiasis is praziquantel, with mass drug administration being the primary means of treatment in many sub-Saharan African countries.

Human Immunodeficiency Virus (HIV) Infection

Anaemia is the most common haematological complication associated with HIV infection in all age groups[62] and has been identified as an independent risk factor for disease progression and death in children. The highest burden of HIV related anaemia is in sub-Saharan Africa with children being the most vulnerable group.[63] Among children aged between 3 months to 18 years in Uganda in 2009, 4.8% of HIV infected children were severely anaemic, and more advanced disease was associated with significantly lower Hb levels ($p<0.0001$). Other significant associated risk factors included younger age ($p<0.0001$) and low CD4 percentage ($p=0.048$).[64] In Malawi in 2008, 6.2% of severe anaemia among children less than five years of age was associated with HIV infection.[63] The mechanism of HIV/AIDS-related anaemia is multifactorial, resulting from HIV infection and the induced anaemia of chronic disease, AIDS-related illnesses, and antiretroviral treatment.[41] Failure of erythropoiesis is the most prevalent mechanism of anaemia in HIV infected children.[63]

Other infectious diseases

Tuberculosis (TB) and bacteraemia have also been associated with severe anaemia in sub-Saharan Africa. Bacteraemia and TB are common opportunistic infections in children with HIV infection and bacteraemia, especially invasive non-typhoidal bacteraemia are commonly associated with malaria infections.[65] In Malawi, 12.2% of severe anaemia cases in children less than five years of age were attributable to bacteraemia.[63] Among children with HIV infection, TB is an independent risk factor for anaemia (OR 3.23, 1.10-9.70). The aetiology of anaemia in TB is likely multifactorial possibly due to increased IL-6 leading to anaemia of chronic illness and micronutrient deficiencies.[66]

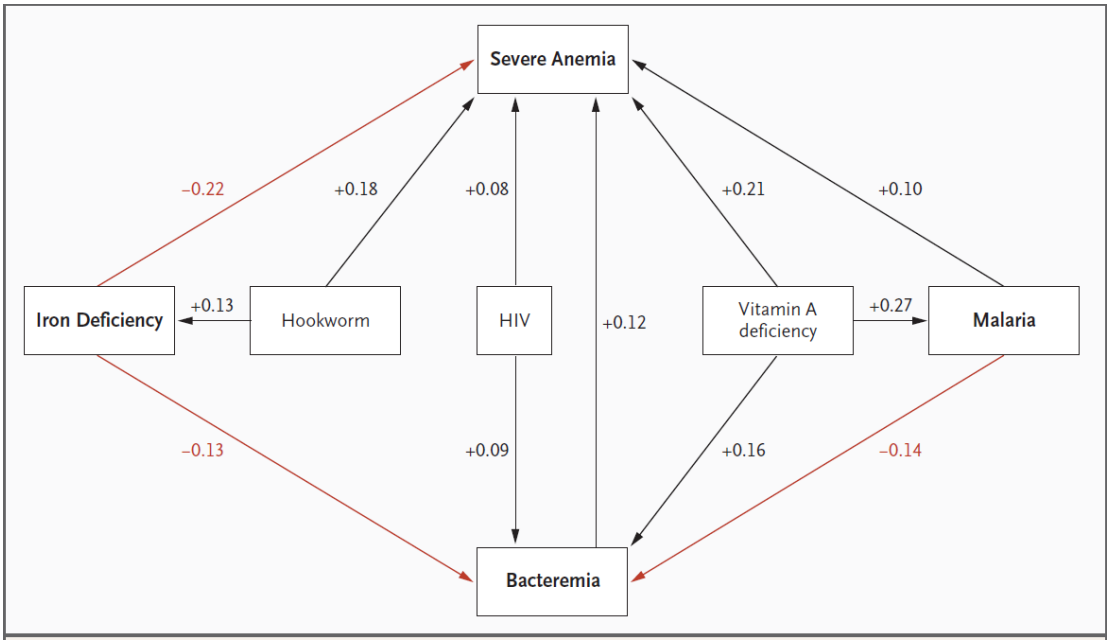
Due to geographic overlap, malaria and visceral leishmaniasis (VL) co-infection may exist, but there is a paucity of data on its prevalence especially in children. In 2006, about 18% of children in a hospital in Uganda had malaria and VL co-infection and 3.4% of patients overall had severe anaemia.[67] VL is less widespread compared to malaria. However,t it remains an important factor to consider in endemic areas in terms of its contribution to severe anaemia.

Genetic haemoglobin disorders

Genetic disorders resulting in structural variations in Hb or reduced production of globin chains of Hb can result in anaemia. In the African region, about 18.2% of the population have a significant Hb

variant.[68] Genetic conditions and haemoglobinopathies such as sickle cell disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency and thalassaemia are associated with anaemia and are common conditions among children under five years of age in malaria-endemic areas because of their partial protection against malaria. In a cross-sectional study among 6-35 months old children in western Kenya, Sickle cell trait (HbAS) and disease (HbSS) were found in 17.1% and 1.6% of children, respectively; 38.5% were heterozygotes and 9.6% were homozygotes for α^{+}-thalassaemia. The Hp 2-2 genotype was found in 20.4% of children, whereas 8.2% of males and 6.8% of children overall had G6PD deficiency.[69] Another cross-sectional study in Uganda among children less than 18 months found a prevalence of 13.2% for HbAS and 0.7% for HbSS.[70] The main mechanism of anaemia in these conditions is intravascular or extravascular haemolysis,[71,72] sequestration of deformed RBCs in the vascular system due to increased adhesion of the red blood cells to the endothelium[73] and RBC production failure (RCPF).[74]

Figure 2: Structural equation model for severe anaemia, iron deficiency, bacteraemia and malaria.



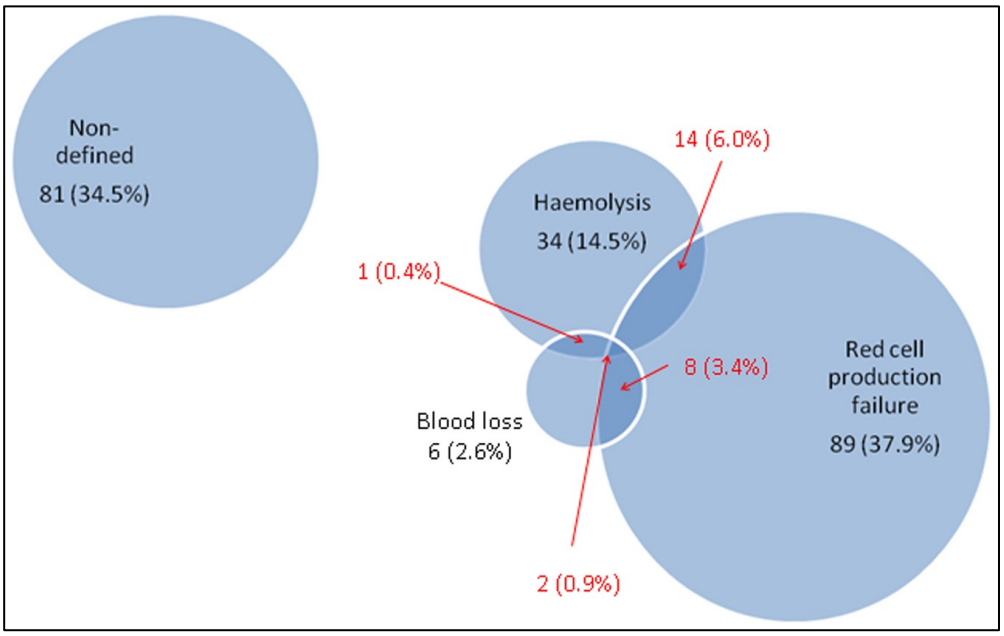
Adapted from Calis et al.[1]

The sizes of significant (P≥0.05) factors associated with severe anaemia are indicated by the standardised regression coefficients (range, −1.0 to +1.0). Inverse (protective) associations are indicated by red lines.

The pathogenesis of severe anaemia is complex and multifactorial. Acute or chronic blood loss through urine (e.g. schistosomiasis), stool (e.g. hookworm) or trauma, haemolysis (e.g. due to

malaria, bacteraemia, haemoglobinopathies), micronutrient deficiencies (e.g. vitamins A and B12) and RCPF (e.g. due to parasitic, bacterial or viral infections) have been documented as mechanisms leading to anaemia.[75,76] These mechanisms act singly or synergistically to reduce the RBC mass or impair with the functionality of Hb (Figure 2). In a Malawian study,[77] RCPF (defined as reticulocytes <50,000/µL and Hb of <5g/dL) was identified as the most prevalent mechanism leading to anaemia with 38.7%-59.7% of patients with haemolysis or blood loss having RCPF overall. For children with severe malaria anaemia, RCPF was identified in 40.2% and haemolysis in 17.2%, however, no mechanism could be defined in 43.7% of the cases (Figure 3). Known mechanisms for malarial anaemia include; RCPF,[77] dyserythropoeiesis,[78] splenic sequestrations of parasitised and uninfected RBCs[79], and lysis of infected and uninfected RBCs.[80]

Figure 3: Pathophysiological mechanisms of severe anaemia syndrome



Adapted from van Hensbroek et al.[77]

Non-defined are mechanisms not fulfilling the definitions for red cell production failure, haemolysis or blood loss. Number and percentages denote mechanism sub-groups not overlapping (black) and overlapping (red).

The burden of severe malarial anaemia

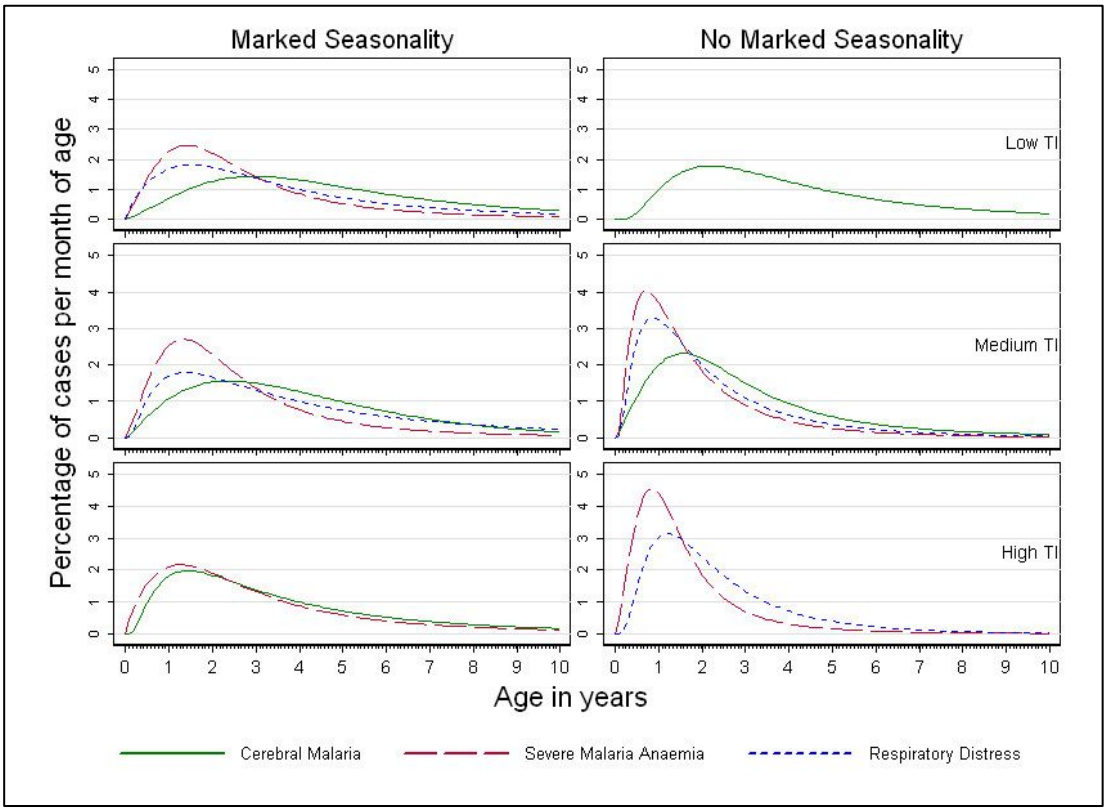
The WHO defines severe malarial anaemia as Hb concentration <5 g/dL or haematocrit <15% in the presence of any level of malaria parasitemia.[20] In high malaria transmission settings, severe malarial anaemia (SMA) is the major presenting syndrome of severe malaria in children aged less than five

years.[30] Other causes of anaemia in malaria-endemic areas of sub-Saharan Africa are common, and malaria infection may exacerbate existing anaemia leading to SMA. Blood transfusion and administration of artesunate are major components of management. Access to a safe and adequate blood supply for transfusion is still a major challenge in developing countries where the burden of malaria is also high.[81]

It is difficult to accurately estimate the prevalence of SMA due to variability in the methods of quantifying malaria burden, difficulties in accurate diagnosis of malaria and the use of presumptive diagnosis in the management of malaria in endemic areas. However, SMA is estimated to affect between 1.5 to 5 million children annually contributing up to half of all childhood deaths from malaria.[5,82,83] In children, the risk of developing SMA generally decrease with increasing age. The risk is high during infancy and increases significantly until the age of two years, and then decrease significantly between four to ten years, this risk is also associated with the degree of malaria parasitaemia with the geometric mean parasitaemia being negatively correlated with Hb concentration.[84] Malaria transmission intensity and seasonality also influence the incidence of SMA with a higher incidence of SMA in children aged 6 months and above in high transmission areas, whereas in moderate to low transmission areas SMA mainly presents in older children and adults as does cerebral malaria.[85,86] In a prospective study in Tanzania, children with severe malaria residing in different altitudes were recruited into a study after admission to hospital. It was observed that the odds of SMA significantly reduced with increasing age ($p<0.001$) and decreasing intensity of transmission (higher altitudes) ($p=0.03$). At one year of age, the odds of SMA was highest in areas with high transmission intensity (lower altitudes) and two years for areas with low to moderate transmission intensity.[87] These findings are consistent with reports from other studies.[30,88] However, a systematic literature review showed younger peak ages for hospitalisations with SMA in areas with uniform all year transmission settings compared to areas with highly seasonal malaria transmission (Figure 4). This pattern was not influenced by the levels of malaria transmission intensity. Therefore the median age of children with SMA remained the same irrespective of the level of transmission intensity.[85] This contrasts with cerebral malaria where the burden shifts towards the younger age groups with increasing transmission intensity, although generally, the median age of patients with SMA is lower than that for cerebral malaria.[86,89] The complex and multifactorial aetiology of anaemia likely masks the underlying relationship between SMA and transmission intensity.

The decline in the burden of SMA with increasing age is likely due to the acquisition of natural immunity, especially in children living in high transmission areas. The rate of acquisition of natural immunity in relation to the degree of exposure is still a controversial issue,[90] but repeat infections are known to potentiate its development.[86,87] However, the age of first exposure to malaria parasites may not affect the rate of acquisition of naturally acquired immunity.[91] A modelling study specifically designed to analyse the relationship between acquisitions of malaria-specific immunity with increasing exposure concluded that physiological changes might also alter symptoms of malaria with increasing age.[92]

Figure 4: Age-patterns of cerebral malaria, severe malarial anaemia, and respiratory distress in Sub-Saharan Africa.



Adapted from Roca-Feltrer et al.[85]

Percentage of cerebral malaria, severe malarial anaemia, and respiratory distress admissions per month of age in children under ten years of age, for each cell of the transmission intensity-seasonality matrix.

SMA mortality is high with wide variations from 1.7%[93] to 23%[30,88,94] in different settings depending on the transmission intensity, seasonality of transmission, age, accessibility to prompt medical care and presence of other underlying medical conditions such as malnutrition and HIV infection. Most SMA associated deaths occur soon after admission partly attributed to challenges in the availability and use of blood for transfusion and other supplies and diagnostic challenges.[30] As much as there has been a decline in malaria attributable deaths over the last 1-2 decades, the case fatality rate for SMA has not changed significantly. In Kilifi, Kenya, the overall case-fatality rate for non-severe malaria was 2.1% from 1990 to 2007 ($p<0.0001$), whereas this fatality rate fell significantly over time from 6.5% to 1.3%, the case fatality rate for SMA remained high (5.9%), and there was no significant fall in the 18 year period (as well as for cerebral malaria).[86]

Children who survive SMA have been shown to have major neurological sequelae later in life.[27]

Severe anaemia and post-discharge morbidity and mortalityThe burden of in-hospital mortality due to severe anaemia is well recognised in malaria-endemic areas of Africa. However, the immediate post-discharge period is a neglected area of research. Several studies have reported the mortality rates in the first three to six months post-discharge to be similar to or higher than in-hospital mortality, likely due to a combination of environmental, behavioural, nutritional and genetic risk factors.36,37,45,95-98

A case-control study in Malawi among 6-60 months old children admitted to hospital with severe anaemia indicated that children admitted with severe anaemia are not only at high risk of dying during the acute phase in hospital but also for several months after they leave hospital; 6.4% of cases died in-hospital and 12.6% died post-discharge. This was significantly greater than in hospital controls (2.9%) or community controls (1.4%) (Log-rank test, $p<0.001$). At six and eighteen months, post-discharge 8.2% and 11.6% of cases were dead compared to 1.6% and 2.7% of hospital controls. Furthermore, 5.9% and 9.1% of cases were readmitted with severe anaemia by six and eighteen months compared with 0.5% and 1.1% among the hospital controls respectively.[45]

In western Kenya, a longitudinal follow-up of children admitted with severe anaemia demonstrated a 16% in-hospital mortality and 10% post-discharge mortality among severely anaemic children in the initial four weeks of follow-up and a further 4% between four and eight weeks post-discharge; children with severe anaemia were at a higher risk of dying (29%) than those without severe anaemia (RR=1.52, 1.22-1.90).[99]

In Uganda, 18 months to 12-year-old children admitted with SMA were followed up for six months post-discharge. 2.1% of children with SMA died, and 0.7% of those with cerebral malaria died post-discharge, with no deaths in the community controls during the same six months' follow-up period.[100]

Malaria as a cause of post-discharge severe anaemia and mortality

Children discharged back to the community in malaria-endemic areas are at risk of another malaria infection or recrudescence due to poor management or drug resistance; thus malaria is likely to be an important contributor to mortality and morbidity in the post-discharge period due to slow haematological recovery, RBC destruction, and RBCPF.[101]

In an observational study in Kenya, malaria was found to be the primary diagnosis in about 33% of children who died after discharge following admission with severe anaemia.[96] In the same cohort, Lackritz et al. showed that malaria parasitaemia in the post-discharge period significantly negated

haematological recovery[99] which is known to take at least four to six weeks and maybe prolonged if there is inadequate parasite clearance or new malaria infections occur post-discharge due to on-going red cell destruction and RCPF.[80,102] In the same study, children who were transfused and did not have malaria parasitaemia had significantly higher Hb levels one-month post-discharge compared to those who were not transfused (least-square means of Hb: 10.2 ± 0.21 versus 9.6 ± 0.15 g/dL, p=0.01). However, the presence of malaria parasitaemia on follow-up negated any beneficial gains from the blood transfusion leading to no difference in the Hb levels between transfused and non-transfused children a month later (8.8 ± 0.23 versus 8.9 ± 0.19 g/dL respectively), and a similar picture was seen two months later.[99] Bloland et al. reported similar findings resulting from inadequate clearance of malaria parasitaemia due to continued use of chloroquine as first-line treatment for malaria in Kenya and Malawi in 1993 despite the widespread resistance to chloroquine.[103]

In Uganda, Opoka et al. reported that among children previously admitted and treated for severe anaemia, significantly more children in this group were readmitted because of malaria (IRR=17.29, 2.02-148.35) compared with the community controls.[100]

Routine management of severe anaemia in malaria-endemic areas

Ideally, the management of severe anaemia should be tailored towards alleviating the acute condition and instituting definitive management based on the underlying aetiology. Standard in-hospital treatment of severe anaemia in many countries in sub-Saharan Africa consists of a blood transfusion and parenteral artesunate for severe malaria.[104] In the case of severe malarial anaemia, this is completed with a 3-day course of artemisinin-based combination therapy (ACT), usually artemether-lumefantrine. Children are often discharged with a short course of iron and folate, typically with no further scheduled follow-up. The national clinical guidelines for the management of anaemia in both Kenya and Uganda advocate for the investigation into the underlying causes of anaemia and appropriate management. However, due to inadequate human and infrastructural challenges in the healthcare systems in these countries, management of severe anaemia is often instituted presumptively following the IMCI guidelines,[105,106] without much further diagnostic investigation. Coupled with poor adherence to WHO guidelines for the rational use of blood for transfusion and the frequent acute shortage of safe blood, these children are likely to be readmitted because of recurrence of severe anaemia or are exposed to a higher risk of post-discharge mortality as has been observed in previous studies.[107,108] This situation is likely to worsen following the recent withdrawal of The President's Emergency Plan for AIDS Relief's (PEPFAR)

support of blood transfusion services in most sub-Saharan African countries. Between 2004 and 2014, a period of consistent-PEPFAR support, there was a tremendous improvement in access to safe and adequate blood supplies and a decrease in the proportion of HIV positive donor blood. However, other transfusion-transmitted infections such as hepatitis B and C have increased in prevalence during the same period.[109] Malaria prophylaxis is recommended for all blood recipients in this setting as routine screening for malaria parasites is not recommended.[110] Due to frequent shortage of anti-malarial drugs and poor adherence to treatment guidelines, it is likely that some children are infected with malaria parasites following blood transfusion.

Guidelines for in-patient management of severe anaemia in Kenya[52] and Uganda[51] are available and strict adherence has been shown to significantly reduce anaemia specific in-hospital mortality.[111] Therefore, training and supportive supervision of clinicians in this setting and the provision of an effective post-transfusion malaria chemoprophylaxis are likely to substantially reduce severe anaemia associated mortality.

WHO recommended malaria prophylactic treatment strategies

Malaria chemoprevention is a potential strategy for preventing new or recrudescent malaria infection in severely anaemic children post-discharge. When it is free from the effects of malaria parasitaemia, the bone marrow gets time to recover, resulting in a more sustained haematological recovery post-discharge.[107]

Intermittent preventive therapy (IPT) could be a potential strategy for the prevention of post-discharge malaria episodes in children diagnosed with severe anaemia in highly malaria-endemic and epidemic regions. IPT is the administration of a full treatment course of long-acting antimalarials at pre-defined time intervals irrespective of a patient's malaria status to clear existing infections and provide prolonged prophylaxis against new infections.[112] Currently, WHO recommends the following IPT strategies for control of malaria.

Intermittent preventive therapy in pregnancy (IPTp)

Malaria in pregnancy is associated with adverse pregnancy outcomes including severe anaemia, low birth weight (LBW), perinatal mortality, abortion, pre-term delivery and death especially in primigravidae and secundigravidae[113] with about 32 million pregnancies at risk in Africa annually.[114] The population attributable risk per cent of post-neonatal infant mortality due to placental malaria has been estimated to be 29% among the primigravidae[115] and reduction in the risk of LBW has

been estimated to reduce neonatal mortality among the primigravidae by 42% and post-neonatal mortality by 18%.[116]

IPTp policies using sulphadoxine-pyrimethamine (SP) were implemented in pregnant women living in areas with endemic malaria transmission as a measure to reduce the adverse impact of *Plasmodium falciparum* malaria in pregnancy.[117,118] Over the past decade the effectiveness of IPTp with SP has been threatened by increasing levels of resistance of *Plasmodium falciparum* to SP.[119,120] Alternative strategies using amodiaquine, mefloquine[121] and azithromycin-chloroquine[122] have been unsuccessful as better replacements for IPTp-SP because of low tolerance, but the use of IPT with monthly dihydroartemisinin-piperaquine (DP) is a promising alternative.[123-125] Despite the high levels of resistance, IPTp-SP still offers substantial protection against adverse pregnancy outcomes in areas where the prevalence of parasites with quintuple *Plasmodium falciparum* dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthetase (*Pfdhps*) mutations is greater than 90%.[114,120] More than 2-doses of IPT during pregnancy has been shown to offer better outcomes than the previous WHO recommendation of 2 doses. A meta-analysis of 7 trials on pregnant women living in sub-Saharan Africa comparing 2-dose to 3 or more monthly doses of IPTp-SP and involving 6281 pregnancies showed that three or more doses of IPT-SP were associated with a higher mean birth weight and fewer low birth weights births since 2012 WHO recommends IPT-SP to be started in the second trimester and given to pregnant women at every antenatal clinic visit until delivery as long as the IPTp-SP doses are given at least a month apart.[118,126,127]

Intermittent preventive therapy in infants (IPTi)

Following the successful deployment of SP for IPTp, studies on IPTi were first conducted around 2000 to take advantage of SP which is available, low-cost, well-tolerated and a long-acting anti-malaria to reduce morbidity and mortality in infants due to malaria in malaria-endemic areas. In 2001, Schellenberg et al. conducted a RCT in Ifakara, Tanzania, to test the safety and efficacy of SP in reducing the rates of clinical malaria and severe anaemia in children less than 1-year-old. The intervention was administered to children at the ages of 2, 3, and 9 months during their routine WHO's Expanded Program on Immunisation (EPI) clinic visits. The protective efficacy (PE) of SP against clinical malaria was 59% (95% CI, 41%-72%) and it reduced the rates of severe anaemia by 50% (PE=50%, 8%-73%) compared to placebo during the first year of life; the drug was safe and well-tolerated.[128] The effects against clinical malaria were sustained for another one year after cessation of SP administration (PE=36%, 11%-53%).[129] Macete et al. did a placebo-controlled trial of SP among 1503 Mozambican children attending EPI clinics between 2002 and 2004 by

administering the intervention at 3, 4 and 9 months of age. During the first year of life, SP was associated with a 22% (3.7%-37.0%, p=0.020) reduction in the incidence of clinical malaria and 19% (4.0%-31.0%, p=.014) reduction in hospital admissions; there was no effect on the incidence of severe anaemia (PE=12.7%, −17.3%-35.1%, p=0.36). The PE against clinical malaria was not sustained in the subsequent year.[129] Between 2004 and 2008 in Kenya, a comparison of SP plus 3 days of artesunate (SP-AS3), 3 days of amodiaquine-artesunate (AQ3-AS3) and 3 days of chlorproguanil-dapsone (CD3) administered during EPI visits at 10 weeks, 14 weeks and 9 months found a PE of 25.7% (6.3%-41.1%); 25.9% (6.8%-41.0%); and 16.3% (−5.2%-33.5%) in the SP-AS3, AQ3-AS3, and CD3 groups, respectively.[130] These studies supported the use of long-acting antimalarials for IPTi. The differences in the efficacy of these trials are likely due to differences in the intensity of malaria transmission, age of participants, levels of *plasmodium falciparum* resistance to SP and the levels of deployment of malaria control interventions especially ITN use.

Pooled data from six RCTs in four African countries involving 7930 infants show that IPTi had a PE against clinical malaria of 30·3% (19·8%-39·4%, p<0·0001); against the risk of anaemia of 21·3% (8·2%-32·5%, p=0·002); against all-cause hospital admissions of 22·9% (10·0%-34·0%, p=0·001) and against hospital admissions associated with malaria parasitaemia of 38·1% (12·5%-56·2%, p=0·007).[131] IPTi-SP for *Plasmodium falciparum* control in Africa is currently recommended by WHO for areas with moderate to high transmission (annual entomological inoculation rates (EIR) ≥10) and where parasite resistance to SP is not high (*Pfdhps* 540 mutation of ≤50).[126] WHO recommends the delivery of IPTi at the same time as the delivery of routine EPI vaccines.

Seasonal malaria chemoprevention (SMC)

Studies conducted, between 2006 and 2011, to evaluate the safety and efficacy of different antimalaria combinations for IPT in children living in malaria-endemic areas with seasonal transmission showed substantial reductions in the incidence of malaria infections, severe malaria, moderate anaemia and all-cause hospital admissions in children aged between 2 to 5 years.[132-135] Two systematic reviews and meta-analysis of studies reporting the safety and efficacy of intermittent preventive treatment in children (IPTc) arrived at similar conclusions. For studies conducted up to 2010, Wilson et al. showed that IPTc conferred a PE of 57% (24%-76%) against all-cause mortality during the malaria transmission season and 82% (75%-87%) overall PE against clinical malaria.[136] For studies up to 2011, Meremikwu reported that IPTc prevented about 74% (Rate Ratio [RR]=0.26; 0.17-0.38) of episodes of clinical malaria, 74% (RR=0.270, 10-0.76) of episodes of severe malaria and 29% (RR=0.71, 0.52-0.98) lower risk of moderate to severe anaemia,

the impact was considerable in both areas with high or low usage of ITNs.[137] Since 2012, WHO recommends SMC (formerly called IPTc) for children 3-59 months old living in areas with highly seasonal malaria transmission throughout the Sahel sub-region using SP and amodiaquine (AQ) in areas where; more than 60% of clinical malaria cases occur in short seasons of ~4 months, SP and AQ efficacy is >90% and malaria clinical attack rate is >0.1 in the 3 -59 months old children.[138,139] SMC with DP is a suitable potential alternative to SMC-SP+AQ. However, currently, SP+AQ is still efficacious in the Sahel and sub-Sahel regions of Africa.[140]

IPT and post-discharge anaemia management

Children who have survived an episode of severe malaria anaemia have a higher risk of suffering another episode, and chemoprevention is a potential strategy. However, currently, there is no policy guiding the use of IPT in children with severe anaemia in the malaria-endemic areas. Several trials on IPT in children diagnosed and treated for mild to moderate anaemia in different malaria epidemiological settings have shown that co-administration of haematinics and IPT improves Hb recovery in anaemic children.

In a RCT trial in 1998 to 2000 among 215 children with moderate anaemia (Hb between 5.0 to 8.0 g/dL) and less than five years of age living in a refugee camp in Tanzania, Tomashek et al. hypothesized that administration of haematinics with a long-acting and effective antimalarial could induce extended periods of aparasitaemia to allow full haematological recovery. The study was divided into three arms, and all participants were on iron and folic acid supplementation. Arm one received a vitamin placebo, and symptomatic participants received chloroquine, arm two received thrice-weekly vitamin placebo and monthly presumptive malaria treatment with SP and arm three received Vit A and C (VAC) three times per week and monthly SP. Participants were followed up for three months. A 3.6 g/dL increase in Hb was reported with no significant differences among the three arms. A significant proportion of participants in arm three had normal iron stores (TfR 8.5 g/mL) compared to arm two (p=0.012), and arm two had lower mean serum transferrin receptor levels than arm one. Therefore, monthly SP and VAC supplementation is more effective in the management of moderate anaemia in participants with iron deficiency anaemia living in areas with a high risk of malaria infection.[141]

Another RCT conducted in an area with intense perennial transmission in western Kenya in 1999, evaluated the efficacy of single and combined therapies with iron supplementation and IPT with SP in improving Hb levels and to assess the risk of malaria infection among 2 to 36 months old

children. A total of 546 participants aged 2 to 36 months with Hb of 7 to 11 g/dL were enrolled, with a single dose of SP and a bed net being issued at enrolment. The participants were randomly allocated into either of the 4 arms a) IPT with SP at four and eight weeks and daily iron for twelve weeks, b) daily iron and IPT with SP placebo, c) IPT and daily iron placebo, or d) daily iron placebo and IPT with SP placebo (double placebo). Compared with double placebo, ITP with SP and iron was most effective in the management of mild anaemia with a mean Hb concentration gain at the end of follow up of 1.14 g/dL (0.82-1.47 g/dL). In terms of improvements in Hb levels, IPT with iron was significantly more effective than iron alone (mean difference [MD] in Hb concentration, 0.35 g/dL (0.03-0.68 g/dL, p=0.03), and IPT alone was not significantly more effective than double placebo (MD in Hb concentration, 0.17 g/dL (-0.15-0.49 g/dL, p=0.30). IPT had modest benefits on haematological recovery, but the incidence of malaria parasitaemia and clinical malaria associated with the use of IPT was reduced by 50%.[142]

In 2007 in The Gambia, Cox et al. evaluated the potential use of weekly chloroquine as a possible intervention to induce haematological recovery in children with moderate post-malarial anaemia. Due to chloroquine resistance, the first-line drug for the treatment of uncomplicated malaria was changed in The Gambia from chloroquine-SP to co-artemether in 2008, thus children were treated with co-artemether before enrolment in the study. They hypothesized that the anti-inflammatory properties of chloroquine could enhance erythropoietic recovery by suppressing plasmodium induced inflammation.[143] Apart from lysis of parasitised and non-parasitised erythrocytes, the pathogenesis of malarial anaemia includes iron sequestration in the macrophage monocyte system and in the hepatocytes as an outcome of inflammatory-induced iron delocalization, a process which reverses when inflammation wanes.[144] Cox et al, randomised ninety-six children aged 12-72 months with uncomplicated malaria to receive either weekly chloroquine (n=50) or placebo (n=46) and followed them up for ninety days. There was no significant difference in Hb change between the two arms at the end of follow-up; CQ +1.04 g/dL (0.67-1.34 g/dL) vs placebo +0.76 g/dL (0.29-1.24 g/dL).[143] In this study, recruitment of participants over 2 malaria seasons with one season of low malaria transmission resulted in lower parasite densities and therefore a lowering of the effect size. The use of low dose chloroquine 5 mg/kg/week, as opposed to the recommended anti-inflammatory dose of 3-4 mg/kg/day may have contributed to the inability to detect an effect of chloroquine on Hb change.

A Cochrane review on the use of intermittent preventive antimalarial treatment for children mainly with mild-moderate anaemia in malaria-endemic areas showed a small increase in Hb levels after

three months of follow up but did not show an effect on mortality and hospital admission.[145] A 0.32 g/dL (MD=0.32, 0.19-0.45) increase in the mean change in Hb from baseline to 12 weeks of follow-up was observed in 4 trials with a cumulative total of 1672 participants. The protective effect of IPT was found to be low in this review, possibly because half of the included trials were conducted in areas with low malaria transmission and in children with mild-moderate anaemia.

However, a RCT conducted between 2006 and 2009 in areas with intense malaria transmission in Malawi involving children with severe malarial anaemia showed the effects of IPT post-discharge (IPTpd) to be significant compared to placebo. Phiri et al. recruited 1414 children under five year of age who were admitted with severe anaemia (Hb <5g/dL) and were randomised to either receive monthly IPTpd with AL or placebo for three months. Phiri et al. demonstrated an adjusted PE of 31%, (5-50) against all-cause mortality or hospital re-admittance because of all-cause severe anaemia or severe malaria between one and six months after enrolment. This effect was more pronounced during the initial three months (PE=41%, 10-62, p=0·01) when the pharmacological effects of AL were active in the system than during the subsequent three months post-intervention (PE=17%, –27-45, p=0·395). The beneficial effect was in addition to the initial effect from the standard AL treatment course provided at discharge and in addition to any protective effect by ITNs.[101] These results are consistent with earlier findings from The Gambia in a study conducted between 2003 and 2004, where children aged between 3 months and 9 years with a Hb concentration less than 7 g/dL were randomised to receive either monthly SP or placebo during the malaria transmission season. The proportion of children with Hb less than 7g/dL at the end of the malaria transmission season was similar between the two groups; prevalence ratio 0.89 (0.44-1.8, p=0.742). However, the PE of SP against recurrence of severe anaemia was 78% and against clinical malaria was 53% (37%-65%).[98] Chemoprevention likely benefits children with severe anaemia more than those with mild or moderate anaemia. These data are scarce but indicate that IPT in the post-discharge period provided to children treated for severe anaemia may potentially provide substantial health benefits.

Other malaria chemoprophylaxis options

Co-trimoxazole chemoprophylaxis

Co-trimoxazole (fixed-dose trimethoprim-sulfamethoxazole) is an inexpensive, broad-spectrum antimicrobial drug that is commonly used in low-income countries. Co-trimoxazole was widely used for prophylaxis before the extensive use of antiretroviral therapy (ART) to reduce morbidity and mortality associated with malaria, Pneumocystis jirovecii pneumonia, bacterial infections, and

diarrhoea among HIV infected adults and children in Africa.[146,147] There is widespread bacterial resistance against co-trimoxazole in sub-Saharan Africa, co-trimoxazole is also an inhibitor of dihydrofolate reductase and has been shown to act as a sulphonamide potentiator and concerns of cross-resistance with SP have been raised.[148] However, the drug still offers a substantial antimicrobial activity to warrant its use as prophylaxis.[149] A RCT in Zambia showed that co-trimoxazole reduced the risk of death by 43% (HR=0.57, 0.43-0.77, p=0.0002) and hospital admission by 23% among HIV infected children who continued using co-trimoxazole prophylaxis for 19 months compared to those who discontinued; most hospitalizations were due to malaria.[150] Co-trimoxazole offers beneficial protection against malaria among HIV infected individuals. In an area with high antifolate resistant plasmodium genotypes in Uganda, co-trimoxazole prophylaxis was associated with a 39% reduction in the incidence of malaria in HIV exposed infants[151] and in another area with similar levels of resistance, co-trimoxazole was associated with a 43% reduction in malaria incidence or 97% when combined with the use of ITNs,[152] similar findings were obtained in Mali.[148] Two systematic reviews concluded that co-trimoxazole is safe, well-tolerated and efficacious for treatment and chemoprevention against malaria in HIV infected adults and children in Africa.[153,154] WHO now recommends that in "children living with HIV in settings where malaria and/or severe bacterial infections are highly prevalent, co-trimoxazole prophylaxis should be continued until adulthood irrespective of ART provision and in settings of low prevalence for both malaria and bacterial infections, co-trimoxazole prophylaxis may be discontinued for children five years of age and older who are clinically stable and/or virologically suppressed on ART for at least six months and with CD4 >350 cells/mm3".[155]

There is limited information about the baseline markers of resistance for both SP and AQ collected in the Sahel and sub-Sahelian regions before the routine implementation of SMC. Currently, there is no routine surveillance for resistance markers for SP and AQ in areas where SMC is being implemented. WHO recommends a baseline assessment of resistance followed by 2-3 years interval surveys in representative locations. The current information on the efficacy and the prevalence of molecular markers for resistance to SP and AQ in SMC implementing areas is only available in research settings. The effects of the widescale implementation of SMC on the development and spread of resistance to SP and/or AQ is yet to be determined.

Intermittent preventive treatment in schoolchildren (IPTsc)

Successful malaria control strategies in the past 2 decades have been associated with a substantial reduction in the overall burden of malaria, but also a shift in the burden towards older

children.[86,156] This is the school going age children of whom the negative effects of malaria in their school performance and sub-optimal attendance is well recognised.[13,157] The benefits of IPTp, IPTi, and SMC have been demonstrated and similar benefits are likely to be achieved if the intervention is extended to include school going children[158]. However, the optimal regimen remains to be determined.

In 2007-2008 in an area of seasonal malaria transmission in Mali, Barger et al. randomised 296 school going children between 6-13 years old to receive two full treatment doses of either SP+AS, AQ+AS or vitamin C given two months apart during the malaria season. SP+AS and AQ+AS reduced the clinical malaria incidence by 66.6% and 46.5% respectively, compared to vitamin C ($p<0.001$). The prevalence of asymptomatic parasitaemia and all-cause sick child clinic visits were reduced significantly in both arms compared to vitamin C, ($p=0.024$ and <0.001 respectively). The rates of anaemia were similar in the SP+AS (17.7%) and AQ+AS (16.0%) at the end of the malaria season and were both significantly lower than for children in the vitamin C arm (29.6%; $p=0.039$).[159]

In an area with the perennial transmission of malaria in western Kenya, Clarke et al. conducted a cluster-randomised placebo-controlled trial involving children aged between 5-18 years in 30 schools. The schools were randomised to receive three courses of SP+AQ or placebo at 4-month intervals to determine the effects of the SP+AQ on the prevalence of anaemia (Hb<11.0 g/dL). SP+AQ reduced the prevalence of anaemia by about half at 12 months (RR=0·52, 0·29-0·93, p=0·028) and enhanced cognitive ability.[160] In Tororo Uganda, children in two schools aged 8-14 years were enrolled into a randomised placebo-controlled trial to evaluate the efficacy, safety, and tolerability of a single course of SP, AQ+SP or DP among school children in reducing the risk of malaria parasitaemia by 42 days from enrolment. DP was the most efficacious with the lowest risk of parasitaemia of 11.7% (7.9%-17.1%), followed by AQ+SP at 44.3% (37.6%-51.5%). There was no difference in the risk of parasitaemia between SP (79.7%, 73.6%-85.2%) and placebo (84.6%, 79.1%-89.3%), $p = 0.22$. AQ+SP and DP were associated with a significant increase in Hb by 42 days; 0.37 g/dL (0.18-0.56) and 0.34 g/dL (0.15-0.53) increase respectively. SP and DP were well-tolerated, but AQ+SP was associated with more vomiting. [161] Another trial in the same area in Uganda randomised 6-14 years old children to either monthly DP, three monthly DP or placebo. Monthly DP reduced the incidence of malaria by 96% (88%-99%, $p<0.0001$) in 12 months, 94% (92%-96%, $p<0.0001$) reduction in asymptomatic parasitaemia and 40% (19%-56%, $p<0.0001$) reduction in the prevalence of anaemia compared to placebo. Three monthly DP reduced the prevalence of asymptomatic

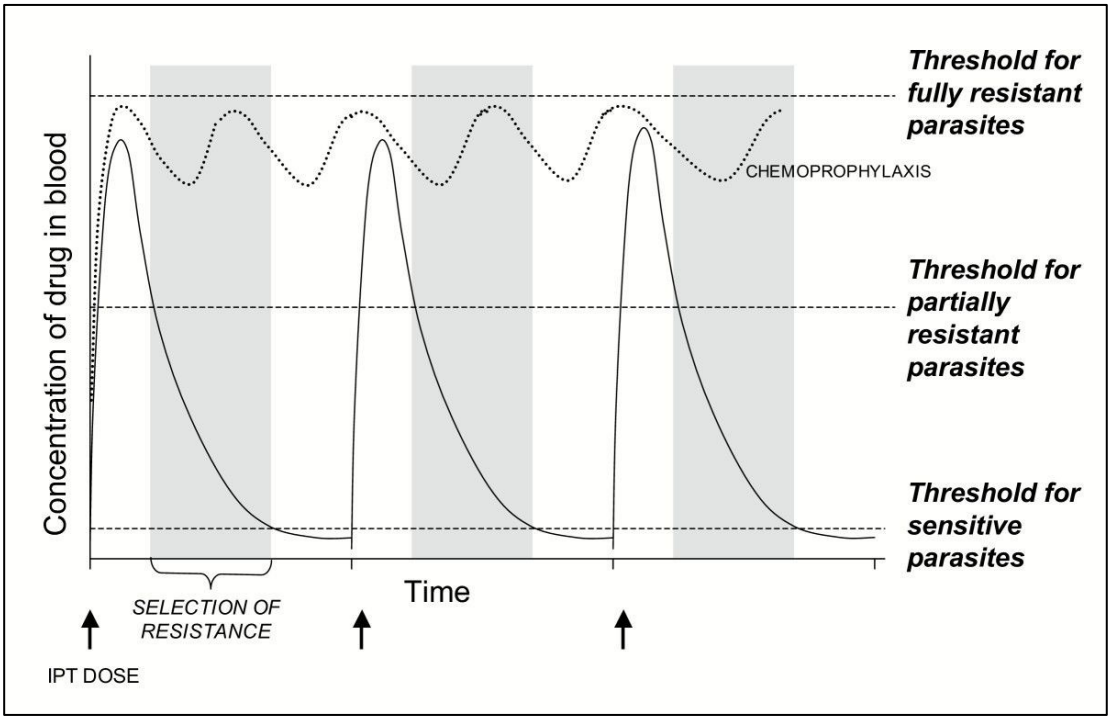
parasitaemia by 54% (47%-60%, p<0.0001), but was ineffective against the incidence of symptomatic malaria or the prevalence of anemia.[162]

These studies demonstrate that an IPTsc regimen using SP monotherapy may not be an optimal option likely due to the widespread *Plasmodium falciparum* resistance to SP across Africa.[163] Monthly DP provides the highest PE against malaria parasitaemia, and clinical malaria and four monthly courses of SP+AQ is also a good alternative. The effects of these regimens on malaria-related anaemia are modest; however, SP+AQ has been shown to have a significant effect compared to placebo.[160,162] IPT is likely to have less impact against malaria-related anaemia in older children as the effects of malaria on Hb decreases with increasing age.[80] Genuine concerns about the spread of drug-resistant parasites and inhibition of the development of naturally acquired immunity to malaria have been raised[158] because the large population of school-aged children, to date WHO has not given policy recommendation for the use of IPTsc.

IPT and risk of development and spread of drug resistance

Development of anti-malarial drug resistance is a major impediment to the fight against malaria, and the possibility of potentiation and spread of drug resistance due to the deployment of IPT is a genuine concern.[158] It is therefore essential to consider the impact of IPT deployment on the spread of drug-resistant parasites and the long-term consequences on the efficacy of the antimalarial drugs used for IPT or chemoprevention. There is usually a gradual decline in the anti-malaria drug concentration in the blood following an initial peak after drug administration. Parasites which are fully sensitive to the antimalarial in use will be cleared by drug concentrations above the minimum parasiticidal concentrations (MPC), and as drug concentration declines parasites with increasing sensitivity will grow, but fully resistant parasites will not be cleared by therapeutic levels of the drug. The window for the selection of resistant parasites is the interval between the drug concentration threshold for partially sensitive parasites and the threshold for sensitive parasites.[164] Resistant parasites will be transmitted to vulnerable individuals when the drug threshold is below the MPC and before the immune system can clear these parasites,[165] this may be more pronounced in children and individuals who are immunologically naïve to malaria parasites and who mainly constitute the infectious reservoir.[166] Other factors that determine the degree of selection pressure for drug resistance include the frequency of drug dosing, the infection status of drug recipients and drugs half-lives; long half-lives lead to longer duration when parasites are exposed to selective concentrations.[167]

Figure 5: Concentration of anti-malarial drug in the bloodstream during IPT



Adapted from O'Meara et al.[168]

SP has been used extensively for IPTp, IPTi, and IPTc in areas with varying levels of malaria transmission intensity. In in-vitro studies, resistance to SP has been shown through demonstration of substitution of alleles within the active site of target enzymes.[169,170] Other methods of establishing drug resistance include; post-treatment analysis of recrudescent infections with the demonstration of the presence of specific mutations at a higher frequency than others[171] or demonstrating the association of specific mutations to treatment failure.[172] The degree of drug resistance is proportional to the number of allelic substitutions that confer parasitic insensitivity to SP with triple-mutant dihydrofolate reductase (*dhfr)* allele being highly resistant in comparison to single or double-mutant *dhfr* allele. The presence of both *Plasmodium falciparum dhfr* triple mutant alleles associated with pyrimethamine resistance N51I + C59R + S108N (CIRN) and *Plasmodium falciparum dhps* double mutants associated with sulphonamides resistance A437G + K540E (SGE) in parasite isolates from an individual is generally associated with SP treatment failure.[78]

Mixed reports have emerged on the impact of IPT on the development and spread of drug-resistant parasite mutants. In a RCT in Senegal conducted between 2002 and 2003 to determine the safety and efficacy of AS+SP combination for use in seasonal IPTi, Cisse et al. observed that the proportion

of parasite positive children with *Plasmodium falciparum dhfr* and *Plasmodium falciparum dhps* mutations were significantly increased during the post-intervention survey compared to the time before the intervention in both the intervention and placebo groups however, the SP+AS group had a comparatively higher proportion of mutants than the placebo group *dhfr:* SP+AS 95%, placebo 75%, p=0.01 and *dhps:* SP+AS 86%, placebo 44%, p=0.001. Since IPTc reduced the overall prevalence of parasitaemia in the SP+AS arm, the prevalence of drug-resistant parasitaemia was lower in the intervention arm compared to the placebo arm (*dhfr:* SP+AS 13%, placebo 28%, and *dhps:* SP+AS 12%, placebo16%). However, at the end of the second year of follow up there were no significant differences in the prevalence of *dhfr* and *dhps* in the intervention and control arms (*dhfr*: SP+AS 88%, placebo 86%, p=0.69, and *dhps:* SP+AS 64%, placebo 77%, p=0.10),[133] similar observations were also reported by Sokhna et al. in 2004 in the same study area using the same drugs.[173]

A RCT conducted in Mali in 2008, Dicko et al. reported a significantly higher frequency of *dhfr*-59 and *dhps*-437 mutations in parasites isolated at the end of the malaria transmission season from children who had received SP+AQ than in those from participants in the placebo arm. The triple *dhfr* mutants and the quadruple mutant (triple *dhfr*+*dhps* 437) associated with significant resistance to SP in children was therefore observed more frequently in the intervention arm than in the control arm.[132] In a study utilizing the same protocol as[132] in 2008 in Burkina Faso, Konate et al. obtained the baseline prevalence of SP and AQ makers of resistance from 3 to 59 months old children from the same community who were not enrolled into the study. At baseline, 32.6% of children carried parasites with *dhfr* mutations at codons 51, 59, and 108 and 25% carried triple *dhfr* plus single *dhps* mutation at codon 437. In comparison to baseline, there was an overall increase in children with parasites carrying the triple *dhfr* mutations only (p=0.001) and triple *dhfr* plus a single *dhps* mutations (p=0.001) in the post-intervention survey. However, there was no significant difference in the proportion of children in the control and intervention arms who carried mutant parasites, and the efficacy of SP+AQ combination was not affected in the year following the intervention.[135]

In contrast to studies conducted in west Africa[132,133,135,173] which reported an increase in drug-resistant mutants to antimalarial drugs, studies conducted in Eastern Africa did not show similar increases. A community randomised trial in Tanzania conducted between 2004 and 2006 to evaluate the effects of IPTi on antimalarial drug resistance reported that IPTi with SP neither lead to a rise in the selection of *dhfr* and *dhps* mutants nor an increase in drug pressure. At the beginning

of this study,[174] SP was used both for treatment of symptomatic infections as well as for IPTi. The prevalence of SP positivity was not significantly different at baseline (in 2004) and 15 months later between the control and intervention arms in both the younger <1-year old (in 2004, p=0.251 and in 2006, p=0.186) or the older ≥1-year-old (in 2004, p=0.34 and in 2006, p=0.38) age groups. Pearce et al. also reported a non-significant difference in the frequency of N51I + C59R + S108-(CIRN) in 2004 (p=0.89) or 2006 (p=0.93) and A437G + K540E (SGE) alleles in 2004 (p=0.52) and 2006 (p=0.54) in both the intervention and control arms. In the final survey in 2007, although there was a non-significant increase in the A437G + K540E (SGE) allele in the intervention than control arms (p=0.18), the N51I + C59R + S108-(CIRN) allele frequency was higher in the intervention than control arm (p=0.004). It is possible, however, that a longer period of follow up could have revealed more changes in the dynamics of drug pressure. Different *dhps* alleles are found in West and East Africa[175], and they confer different levels of resistance to antifolates and may partly be a reason for the differences between this study[148] and the previous studies[132,133,135,173] in West Africa.

Simulated mathematical modelling studies have been designed to address the impact of IPT on the selection pressure for parasite drug resistance. Alexander et al. designed a model simulating malaria transmission dynamic of a rural Southern Tanzanian situation to predict the impact of IPTi with SP on selection pressure for parasite drug resistance. The relative difference in the reproductive success for resistant parasites (in comparison with drug-sensitive parasites), which determines the rate of spread of drug-resistant genotypes, defined the selection coefficient used in the model. The model parameters were; parasite prevalence in different ages, the prevalence of previous antimalarial usage (background dosing rate) and the proportion of individuals not in the IPT population infected with malaria and treated with anti-malarial drugs. The model showed that IPTi would increase the selection pressure for drug resistance by 4.4% and was unlikely to shorten the useful life of the drug used. However, expanding the age of children getting this intervention to 5 years would increase the selection pressure to 31%. High prevalence of malaria parasitaemia and younger age were strong predictors for high selection pressure.[176] The findings of this study were fairly consistent with an earlier composite model designed by O'Meara et al.[177] This model took into consideration the frequency of antimalarial drug use, drug pharmacokinetics, degree of antimalarial immunity and transmission intensity. It was predicted that in areas with low or unstable transmission, IPTi would hasten the spread of both partially and fully resistant parasite mutants, but not in areas where partial resistance was already established. Both[176] and[177] predicted that there is a high likelihood of transmission of fully resistant parasites in high transmission areas. Use

of the same drug for the treatment of true infections (symptomatic individuals) as well as for IPT would accelerate the spread of fully resistant parasites in this setting.[178]

More recently Teboh-Ewungkem et al. modified the initial model[177] by adding the effects of the spatial structure of a population with a focus on the parasite fitness function to determine the effects of IPTi on the spread of antimalarial drug resistance in areas with population movements between low and high malaria transmission settings.[179] This model showed that when areas with different rates of malaria transmission (low and high) are connected, the demography of infection shifts over time in the low transmission areas to mirror the demography of high transmission areas. Eventually, the fully resistant parasites spread throughout the two transmission zones. When the rate of movement between the two regions is high and the time spent is shorter, the two regions will have a strong transmission interaction; the relative rate of spread of partially susceptible parasites relative to fully susceptible parasites and fully resistant parasites relative to partially resistant parasites will be indistinguishable between the two transmission zones. Therefore, the proximity of areas with different transmission rates and the degree human movement activities has an impact in deciding on the antimalarial drug resistance monitoring policy to be used in IPTi.

IPT and effects on the development of natural antimalarial immunity

Partially resistant parasites survive as drug concentration declines between IPT doses, therefore permitting infection and the development of parasite-specific immunity.[168] Naturally acquired immunity against malaria develops cumulatively, and each subsequent infection confers an increasing level of protection against severe malaria. Concerns have been raised about the potential effects of IPT to interfere with/or negate the development of malaria parasite-specific immunity, therefore, leading to rebound of severe clinical disease and mortality during the period immediately following cessation of IPT doses if transmission and exposure levels remained high.

Earlier studies conducted to determine the safety and efficacy of malaria chemoprophylaxis in children demonstrated mixed results of rebound malaria in the transmission period following termination of chemoprophylaxis.[180-182] In 1995 in Tanzania, Menendez et al.[180] conducted a randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis with pyrimethamine-dapsone (Deltaprim) to determine their efficacy in preventing severe anaemia and malaria in children. Eight hundred and thirty-two children were enrolled in the study. Passive case detection and a cross-sectional survey were used to evaluate the incidence of severe anaemia and occurrences of malaria. Compared to placebo, malaria chemoprophylaxis conferred a PE of 57.3%

(43·0-67·9) against anaemia and 60.5% (48·2-69·9) against malaria. However, a rebound effect was observed in the year following the termination of treatment, with children who had received chemoprevention being at a higher risk of severe anaemia RR=2.2 (1.3-3.7) and malaria RR=1.8 (1.3-2.6) compared to children in the placebo group. In another malaria chemoprophylaxis study in 1999 in Mali, Coulibaly et al.[181] conducted a randomised cohort study among persons aged between 3 months to 20 years. Participants were randomly assigned to receive either SP or placebo at the start of the high malaria transmission season. The median time to the first clinical episode of malaria was delayed in the SP group by 29.5 days and the incidence in the first month was reduced to 3% from 26%. However, later evaluation at 24 weeks post-intervention showed that the SP group had an increase in the incidence of the first episode of malaria to 42% compared to 17% in the placebo group. In Contrast to the studies above,[180,181] Greenwood et al. enrolled participants and followed them up for five years in The Gambia. Participants aged three months to 5 years were randomised to 2 weekly pyrimethamine-dapsone or placebo for a maximum duration of 5 years. During the intervention period, malaria episodes dropped by 65% after three years of intervention. However, in the year following the termination of treatment five years later, there were 52% more episodes of malaria in the intervention arm compared to the placebo arm. Although the intervention arm reported an overall reduction of mortality by 15% during the intervention period, there was a higher risk of death in the year immediately following termination of treatment in the 5 to 6-year-old children[183,184]. These trials suggest that continuous and prolonged chemoprophylaxis impairs the development of naturally acquired immunity against malaria.

In contrast to the above studies on chemoprophylaxis, trials conducted to determine the safety and efficacy of various drugs for use in IPTi or IPTc have mostly reported no or statistically insignificant rebounds in malaria in the post-intervention period. In the seasonal IPT study cited above,[133] 85% of the children who were initially recruited into the study were re-recruited and followed up to the end of the subsequent transmission period. An incidence ratio of 0·98 (0·82-1·17) was reported with no significant evidence of rebound of malaria in the intervention group. In a RTC conducted between 2005 and 2006 in Ghana on IPTc, Kweku et al. individually randomised 3 to 59 months old children to receive 6-month courses of either placebo or AS+AQ combination monthly or bi-monthly, or AS+SP combination bi-monthly. The post-intervention analysis showed that the incidence of malaria parasitaemia in the intervention groups among children who were >1-year-old was higher, but not statistically significant compared to the placebo group. However, among those who were < 1 year old when they received monthly AS+AQ, there was a significant increase in the

incidence of malaria.[184] These findings contradict the results reported by Cisse et al. from a 2002-2003 Senegalese study[133] where children who had received intervention in their first year were better protected in the post-intervention period compared to older children. Guinovart et al.[91] conducted a randomised controlled trial between 2005 and 2009 in Mozambique to determine the effects of age and parasite exposure on the development of naturally acquired immunity in infants. Three hundred forty-nine children were randomised into early exposure group (placebo from 2.5 to 4.5 Months and SP+AS from 5.5 to 9.5 Months) or late exposure group (SP+AS from 2.5 to 4.5 Months and placebo from 5.5 to 9.5 Months) and the control group was put on placebo from 2.5 to 9.5 Months. Passive follow-up was continued until the children were two years old. There was no significant rebound of malaria episodes, but those in the intervention arms were found to have a higher, but statistically insignificant increase in the risk of malaria in the second year of life; HR=1.35 (0.81-2.24, p=0.743) and HR=1.38 (0.83-2.28, p=0.642) in the early and late exposure groups respectively. Age may have a bearing on the acquisition of antimalarial immunity; however, this study did not find any evidence to conclude that the acquisition of naturally acquired immunity is dependent on the age of first exposure to malaria parasites. The different findings in these studies could possibly be due to the differences in the transmission intensities, seasonality of transmission and the duration and frequency of the interventions. A random-effect meta-analysis conducted on[132,133,185] to determine the effects of IPTc on rebound clinical malaria in the post-intervention period reported a summary effect measure of 1.11 (0.99-1.24, p=0.07).

In a pooled analysis cited in 0 above, and involving six RCTs[128,129,186-189] on the safety and efficacy of IPTi using SP, the incidence of clinical malaria, anaemia or all-cause hospital admissions were analysed in the five months following termination of IPTi interventions. Although there were inconsistencies between trials, the pooled effect estimate was non-significant. Thus the combined effects of the intervention were not associated with a rebound in the incidence of clinical malaria in the follow-up period.[131] This is consistent with a study in Uganda where Bigira et al. conducted a RCT to determine the PE and safety of monthly SP, or monthly DP or trimethoprim-sulfamethoxazole (TS) in 393 children aged six months of age for 18 months. A year after termination of the intervention, the incidence of malaria in the intervention arm was similar to that in children who received no intervention suggesting no apparent impairment in the development of naturally acquired immunity.[190]

The efficacy of SP has declined over time due to the widespread resistance of the plasmodium parasites to SP, especially in eastern and southern Africa.[191] WHO still recommends the use of SP

for IPTp in this epidemiological setting for lack of better alternative regimens. However, SP is still efficacious in west Africa where it is used for both IPTp and SMC.[139] Other drug regimens such as dapsone and mefloquine may have good efficacy, but have not been recommended for chemoprevention because of low tolerance and / or potential toxicity in apparently healthy individuals, who are the main targets for malaria chemoprevention.[192]

The available body of evidence concerning the effects of malaria chemoprevention in children <5 years old on the impairment of antimalarial immunity is mixed. Overall, however, most studies concluded that there was no significant interruption in the acquisition of antimalarial immunity. Age, transmission intensity, frequency and duration of chemoprevention and the half-live of the drug used (and type of intervention; intermittent, e.g. IPTi vs complete prophylaxis, e.g. SMC) do have some effects of the effectiveness of the intervention as well as the degree of rebound effects after the termination of treatment. In the IPTi and IPTc studies that reported some level of rebound in malaria, they also noted that beneficial effects of chemoprevention were not outweighed by rebound malaria. However, there is a need to consider the effects of chemoprevention in the periods immediately after the termination of interventions.

Experience with dihydroartemisinin-piperaquine for IPT

DP is one of the five ACTs recommended by WHO for the treatment of uncomplicated malaria including AS+MQ, AS+SP, AL and AS+AQ.[193] DP is a very effective antimalarial with cure rates of ≥95% in different epidemiological settings in Africa.[194-196] A systematic review and meta-analysis showed that DP is superior to AL and is as effective as AS+MQ in preventing the recurrence of falciparum malaria parasitaemia.[197] The piperaquine component has a long half-life of 23 days (19-28 days) in adults and 14 days (11-18 days) in children,[198] making DP an attractive candidate for IPT. The half-live of DP is comparable to that of mefloquine and amodiaquine-artesunate combinations in children, but longer than that for SP (4-11 days)[199] and AL.[200] A recent systematic review to evaluate the safety of DP when used for IPT showed that DP, when administered monthly, has a lower risk profile compared to daily TS, monthly SP or placebo.[201]

Because of its long post-treatment prophylactic effects[202] and safety profile, some studies have evaluated its safety and efficacy for use in IPT. In Burkina Faso, Zongo et al. conducted a RCT to evaluate the safety and efficacy of DP as a possible alternative to SP+AQ for use in SMC. Overall, 1499 children aged between 3 and 59 months were randomised to either DP or SP+AQ over three months during the malaria transmission season. Compared to a control group involving children

who did not receive any intervention, both regimens were highly efficacious against clinical malaria at 77% (67%-84%) and 83% (74% to 89%) for DP and SP+AQ respectively.[140] In western Africa, where SP is still highly efficacious, DP has comparable safety and efficacy profiles as SP+AQ or SP+PQ when used for SMC. In The Gambia, a RCT by Bojang et al. found that the incidence of malaria at the end of the malaria transmission season was 0.10 cases per child year (0.05-0.22), 0.06 (0.022-0.16) and 0.06 (0.02-0.15) for DP, SP+AQ and SP+PQ arms respectively. Combination of two long-acting antimalarials in SP+AQ or SP+PQ combinations were more efficacious than DP of which only piperaquine is long-acting. The mean Hb concentration was also comparable in the three arms (10.1 g/dL for SP+AQ, 10.4 g/dL for DP and 10.1 g/dL for SP+PQ).[203]

In East Africa where there is high resistance to SP, monthly DP has been shown to be more efficacious and well-tolerated compared to DP dosed every two or three months, SP alone or in combination with AQ for IPTsc.[161,162] In Uganda, 4-weekly dosing for DP was found to be more efficacious against symptomatic malaria (incidence 0·018 episodes per person-year) compared to 12-weekly dosing (incidence 0·39 episodes per person-year); adjusted incidence rate ratio [aIRR] 0·041, 95% CI 0·012-0·150, p<0·0001), and this protection was sustained for up to one year after the intervention was stopped (aIRR 0·62, 0·40–0·95, p=0·028).[204] DP is also a promising alternative to SP for use in IPTp or intermittent screening and treatment in pregnancy, possibly if used with rapid diagnostic tests with better sensitivity than is currently available.[123]

DP and QT interval prolongation

One of the major side effects of DP is its dose-related prolongation of the QT interval caused by the piperaquine component. [205] It is therefore not recommended in persons with congenital long QT syndrome (CLQTS). This is an arrhythmogenic disease of genetic origin caused by mutations in genes encoding ion channels involved in the control of ventricular repolarisation and is associated with sudden death. There is a paucity of data on the prevalence of CLQTS in the African population, but it is about 1:2534 (1:1583-1:4350) in apparently normal live births in the Caucasian population.[206] Drugs that cause direct blockade of the rapidly activating potassium ion channels may induce QT interval prolongation because of delays in ventricular repolarisation.[207] Drugs which have been shown to be associated with QT prolongation and are contraindicated when taking DP include; antiarrhythmics, e.g. amiodarone and quinidine, antimicrobial drugs such as macrolide antibiotics and fluoroquinolones, non-sedating antihistamines, e.g. terfenadine and anti-malarial drugs such as mefloquine, halofantrine, lumefantrine and quinine.[205,208] Administration of DP with a high fatty meal has been shown to increase its bioavailability and subsequent risk of QT interval

prolongation.[205,209] In rare cases, the drug-induced long-QT syndrome may potentiate the development of '*torsade de pointes'*, an atypical type of polymorphic ventricular tachycardia leading to sudden death.[210] Majority of CLQTS or acquired QT prolongation are clinically silent and may only be diagnosed when electrocardiograms (ECG) are conducted for other reasons. A recent systematic review estimated the risk of sudden death due to DP intake as 1 in 757 950 (1 in 854 490-1 in 209 114) and was not higher than the baseline rate of sudden death of between 1 in 714 280 to 1 in 100 835 of the reference population after standardisation to 30-day risks.[211]

To date, only one death regarded as potentially related to DP has been reported, an apparently healthy 16-year-old woman died suddenly hours after taking the 2nd dose of the first course of DP during a mass drug administration exercise in Mozambique.[212] Clinical trials on DP that have conducted QT interval monitoring by ECG are scarce, but the few have reported minor, clinically silent QT interval prolongation with no significant differences to comparator antimalarial drugs. In Cambodia, a RCT to evaluate the protective efficacy of a compressed (180 mg dihydroartemisinin and 1,440 mg piperaquine) four, monthly 2-day treatment courses of DP was terminated early due to safety concerns. An unblinded review showed that the mean QTcF (using Fridericia's formula for correcting for heart rate) in the DP arm was 46 ms higher than the placebo arm.[213] A systematic review by Zani et al. to determine the safety and efficacy of DP for treatment of uncomplicated malaria found that DP was associated with an increased frequency of prolongation of the QTc interval than mefloquine-artesunate and no difference was seen in prolonged QTc between DP and AL. No cardiac arrhythmias, sudden deaths or deaths attributed to DP were reported in this review.[197] An open-label RCT in Asia reported a statistically significant difference in the proportion of patients having borderline QTcF prolongation (431-450 ms) between DP (13.0%) and AS+MQ (5.3%) ($p<0.001$) and prolonged QTcF (> 450 ms) of 4.7% vs 5.3% for DP and AS+MQ respectively ($p<0.001$). These differences disappeared by day 7 of treatment. An increase of QTcF >60 ms from baseline was reported in 4.6% and 2.9% for DP and AS+MQ respectively ($p<0.001$) and a mean increase from baseline to day 2 of 22.93 ms and 14.65 ms respectively ($p<0.001$) and these had fallen to 10.47 ms and 13.39 ms by day 7 for DP and AS+MQ respectively ($p=0.075$). No cardiac arrhythmias or deaths were reported in this study.[214] Another study by Bassat et al. conducted in 5 African countries reported that there was no significant difference in the borderline (431-450 ms) and prolonged (>450 ms) QTcF interval between DP group (1.0% and 0.2%) and the AL group (1.2% and 0.2%) respectively ($p=0.76$). About 2.7% of participants in the DP and 2.0% in the AL groups had a QTcB (using Bazett's formula for correcting for heart rate) increase of ≥60 ms between day 0 and

2 of drug administration. Two deaths occurred during the study and both were attributed to either severe anaemia or septicaemia.[202] A post-licensure safety evaluation of DP in Ghana involving 444 participants age <6 years conducted ECGs on participants at baseline, before dose three and repeated 3-4 hours post-dose three and on day seven after enrolment. Significant changes were reported in the mean QTcF compared to baseline; day 3 pre-dose -14.5ms (-16.3 to -12.7), day 3 post-dose -28.2ms (-30.3 to 26.0) and day-7 post-enrollment -4.5ms (-6.1 to-2.8). Clinically insignificant QTcF prolongation of >60ms from baseline was reported in 7, 38 and one participant on day-3 pre-dose, day-3 post-dose and on day-7 post-enrollment, respectively.[215]

QTcF prolongation returns to normal levels after 11 to 48 hours after the last dose of DP[205] suggesting that repeat monthly doses of DP if used for IPT is unlikely to result in dose accumulation and increased risk of QTcF prolongation. Studies that have assessed the cumulative effects of DP repeat doses report no increase in QTcF in subsequent doses compared to the first.

An interventional study in Papua New Guinea enrolled 3 to 60 years old apparently healthy individuals and put them on standard 3-day treatment courses of DP over three consecutive months. ECGs were conducted pre-dose and 4 hours after the 3rd dose of each monthly course of DP. There was no evidence of cumulative cardiotoxicity in this study; the mean QTcF increase was 19.6 ms (standard deviation [SD]=17.8 ms) and 17.1 ms (SD=17.1 ms) for the first-course and third-course post-dosing ECGs, risk difference -2.4 (-6.9 to 2.1, p=0.285), respectively. No participant had QTcF of >500 ms, and 3 (4.3%) and 2 (2.9%) of participants had a QTcF prolongation of >60 ms between baseline and last doses of the first and the third courses (p=1.00), respectively.[216] In a sub-study in the trial by Bigira et al[202] 183 ECGs were conducted to monitor the effects of repeat monthly doses of DP on QT interval prolongation. A mean QTc of 396msec (range 278-444, SD=31.3) was recorded on follow up, and none was >450ms. There were no differences in the mean (SD) QTc intervals measured 4-6 hours post-dose 3 for children who had been prescribed 3-5, 6-10, or 11-18 prior doses of DP: Mean (SD) QTc 405 (26), 388 (33) and 396 (33), respectively (Dorsey, unpublished data). Thus, the available data suggests an absence of cumulative cardiotoxicity with repeated dosing in asymptomatic individuals. However, the electrocardiographic safety of monthly DP when provided as post-discharge chemoprevention may require further confirmations as children with severe anaemia may have other risk factors making them more vulnerable to QTc prolongation such as electrolyte disbalances.

The emergence of artemisinin drug resistance

Tremendous efforts have been made in the management of malaria, but further initiatives for control and elimination are threatened by the emergence of artemisinin-resistant *Plasmodium falciparum* which was first reported in western Cambodia.[217,218] It is in the same region where resistance to SP and chloroquine emerged before spreading to the rest of Asia and eventually Africa.[219] Artemisinin resistance is characterised by the prolonged time to parasite clearance,[220] persistence of parasitaemia 72 hours after treatment,[221] re-emergence of parasites within 28 days[222] and reduced in vitro susceptibility to dihydroartemisinin in the presence of adequate plasma concentrations of dihydroartemisinin which indicates reduced susceptibility of ring-stage parasites.[223] In resistant parasites the cell cycle is arrested by dihydroartemisinin but resumes when the drug levels wane. Artemisinin drug resistance is associated with single nucleotide polymorphism in the "propeller" region of the *Plasmodium falciparum* kelch protein gene on chromosome 13 (kelch13).[224]

Artemisinin resistance has now spread to the entire Southeast Asia region. In the Thailand-Myanmar border, the mean parasite clearance half-live was found to be 3.7 hours (3.6-3.8) in 2010 compared to 2.6 hours (2.5-2.7) in 2001 while in western Cambodia it was estimated to be 5.5 hours (5.2-5.9) between 2007 and 2010; the proportion of slow clearing infections concurrently increased.[225] The efficacy of AS+MQ has also been reported to be decreasing in this region,[226] but the prolongation of treatment with ACTs is still an option for the management of such cases.

A multi-country prospective study conducted between 2011 and 2013 to determine the spread of artemisinin-resistant *Plasmodium falciparum* showed that resistance is now prevalent in mainland southeast Asia with no evidence of spread to Africa. Day 3 post-treatment parasitaemia ranged from 0 to 4% in Africa and was as high as 68% in Asia. There were no significant differences in the median parasite clearance half-life in Kenya 2.8 hours (0.9-4.2) and Nigeria 2.6 hours (1.4-7.1) ($p=0.75$) and were significantly shorter in the Democratic Republic of Congo 1.9 hours (0.7-7.0) compared to Kenya and Nigeria ($p<0.001$); most sites in Asia had median parasite clearance of >5 hours.[227]

In addition to artemisinin resistance, the emerging resistance to partner drugs in artemisinin-based combination therapies could further derail efforts in the fight against malaria. The piperaquine 50% inhibitory concentrations (IC_{50s}) was reported to increase significantly in Cambodia between 2011 and 2013 and two markers of resistance to piperaquine; exo-E415G and plasmepsin 2–3 markers

have been identified to be associated with DP treatment failure in Cambodia.[228] In a prospective cohort study in Cambodia, 2-65-year-old participants with uncomplicated *plasmodium falciparum* malaria were treated with DP and peripheral parasite densities measured weekly until 63 days to determine recrudescence. Piperaquine plasma concentrations were measured at baseline, 7 days, and on the day of recrudescence. A higher prevalence of kelch13 mutations and higher piperaquine IC_{50} was found in those with recrudescent infections.[229] Another study also found Increases in treatment failures, parasite clearance times, and piperaquine IC_{50} values.[223] In Cambodia, the resistance to DP is as a result of resistance to both artemisinin and the piperaquine components and the presence of validated kelch13 mutations and amplification of *plasmepsin-2* have been demonstrated.[223,229,230]

Patent gametocytaemia is correlated with the parasite clearance half-life, and the prevalence of pre-treatment and post-treatment gametocytaemia is higher in areas with the increased treatment failure rate. Therefore, artemisinin-resistant *Plasmodium falciparum* infections may have a transmission advantage.[227,231]

Currently, artemisinin-resistant *Plasmodium falciparum* has not been reported in Africa.[232,233] Piperaquine associated plasmepsin-2 gene has been isolated in the west and east Africa, but the drug is still highly efficacious.[230] However, the thread to DP resistance is real as previous resistance to chloroquine and SP were imported to Africa from Asia.[234] Continued surveillance and monitoring for the emergence of resistance is, therefore, a priority in the fight against malaria. The proportion of children treated for severe anaemia and which would require post-discharge malaria chemoprevention is low compared to the overall malaria cases treated with ACTs. Therefore, PMC is unlikely to increase the selection pressure for drug resistance.[176]

The rationale for Post-discharge Malaria Chemoprevention trial

Children recently treated for all-cause severe anaemia are at a high risk of morbidity and mortality in malaria-endemic areas, and malaria has been implicated as a major contributor. Currently, no strategy specifically addresses this high-risk period, and a proactive approach could offer substantial public health gains. IPTp, IPTi and SMC have been deployed successfully and a similar strategy targeted on this high-risk post-discharge period is likely to be accepted by policymakers in malaria-endemic areas. Like SMC, PMC aims at complete prophylaxis for full haematological recovery to be achieved during the intervention period. The previous trial in Malawi[101] showed that three months of post-discharge malaria chemoprevention using three full treatment courses of AL

prevented 31% (95% CI 5-50, P=0.02) of deaths or readmissions due to severe anaemia or severe malaria by six months post-discharge. These results are impressive and needed a further confirmatory trial before the strategy can be considered for policy by WHO and malaria control programs in endemic countries in Africa. PMC aims to determine if 3 months of post-discharge malaria chemoprevention with monthly 3-day treatment courses of DP is safe and superior to the standard single 3-day treatment course with AL provided as part of standard in-hospital care in reducing all-cause readmissions and deaths by 6 months in the post-discharge management of children less than 5 years of age admitted with severe anaemia. DP was chosen instead of AL for PMC because it is now registered as a second-line treatment for malaria in Kenya and Uganda and has higher efficacy and longer post-treatment prophylactic period than AL.

Research Aim and Objectives

Aim

The general aim of this thesis is to generate the necessary evidence required by WHO and Malaria Control Programs in Africa to review whether PMC should be recommended as a strategy for the post-discharge management of children with severe anaemia.

Specific objective 1

To conduct a retrospective cohort analysis of 5 years historical data (2008 to 2013) for children under five years of age from the KEMRI/CDC HDSS who were admitted with severe anaemia or other syndromes to the Siaya County Referral Hospital in Western Kenya, in order to determine the rates of post-discharge mortality.

Specific objective 2

To conduct a meta-analysis of the burden of anaemia and post-discharge mortality and morbidity in children under five years of age in areas with moderate to high malaria transmission intensity in Africa order to provide the necessary data required to estimate the impact of PMC if adopted

Specific objective 3

To determine if 3 months of post-discharge malaria chemoprevention with monthly 3-day treatment courses of dihydroartemisinin-piperaquine (DP) (PMC-DP) is safe and superior to the standard single 3-day treatment course with artemether-lumefantrine provided as part of standard

in-hospital care in reducing all-cause readmissions and deaths by 6 months in the post-discharge management of children less than 5 years of age admitted with severe anaemia.

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Chapter 3: Post-Discharge Mortality Risk in Western Kenya

The Post-Discharge Risk of Mortality in Children Under Five Years of Age Living in A High Malaria Transmission Area in Western Kenya: A Retrospective Cohort Study

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Abstract

Introduction

Severe anaemia is a major public health problem in malaria-endemic areas in sub-Sahara Africa with up to 34% of paediatric admissions with severe malaria also suffering from severe anaemia. Rates of in-hospital mortality are high, and studies show that these children are vulnerable to further mortality after discharge. There are no policies to address this risk period, and a better understanding of the burden and risk factors for post-discharge mortality are needed. We aimed to determine the risk of post-discharge mortality among children <5 years of age admitted with all-cause severe anaemia or other syndromes in western Kenya.

Methods

We conducted a retrospective cohort analysis using data previously collected from continuous paediatric in-hospital surveillance and population-based Health and Demographic Surveillance Systems in Siaya County, western Kenya. The data was analysed using Cox regression survival analysis and Mantel-Haenszel odds ratio (MHOR) for paired binary outcomes. The primary outcome was all-cause mortality in the first six months post-discharge.

Results

Between January 1, 2008, and December 31, 2013, inclusive, 3,639 hospital admissions involving 4,423 different diagnoses were recorded. The primary diagnosis included: severe anaemia (n=655), severe malaria (n=1033), pneumonia (n=996), severe acute malnutrition (n=271) and 'other syndromes' (n=1,468). Overall, in-hospital mortality was 2.8% (101/3639) and the post-discharge mortality by three, six and twelve months among the 3538 survivors was 7.6%, 9.4%, and 12.4%, respectively. Comparison of six-month post-discharge mortality in children with versus without specific syndromes showed that children hospitalised with severe anaemia had higher six-month post-discharge mortality than other children without severe anaemia (HR=2.46, 1.69-3.56, p<0.001). Similarly, severe acute malnutrition was associated with higher six-month post-discharge mortality (HR=3.86, 2.56-5.81, p<0.001). Severe malaria, without severe anaemia, was associated with lower post-discharge mortality than children without severe malaria (HR=0.38, 0.22-0.67, p=0.001). Children admitted with severe anaemia also had significantly higher odds of six-month post-discharge mortality than in-hospital mortality (MHOR=2.02, 1.19-4.05, p=0.011). Younger age, lower socioeconomic status, living far from the hospital, lower maternal education and maternal HIV-positivity were associated with 6-month post-discharge mortality.

Conclusion

The first six months post-discharge is a high-risk period for mortality among children admitted with severe anaemia and severe acute malnutrition in a malaria-endemic area in western Kenya. Strategies to address this risk period are needed.

Introduction

Severe anaemia is a major public health problem in malaria-endemic areas in sub-Sahara Africa and has a complex and multifactorial aetiology that includes nutritional factors, and acute and chronic infections such as malaria, tuberculosis and Human Immunodeficiency Virus (HIV) infection.[1] In young children in sub-Saharan Africa, up to 34% of paediatric admission with severe malaria[2-4] also have severe anaemia.

Rates of in-hospital mortality due to severe anaemia range from 4 to 12% in different epidemiological settings[5-7] and can be as high as 16% in the combined presence of respiratory distress and malaria.[8] However, several studies have shown that the first few months after discharge is also a high-risk period for further mortality, which can be as high, or higher than in-hospital mortality,[9-11] particularly in malaria-endemic areas, possibly reflecting the effects of new or recrudescent malaria infections.[11,12]

The high risk of post-discharge mortality has received little attention relative to the burden, diagnosis and care of children during the in-hospital stay,[13] and there are currently no specific policies to address the post-discharge period. An improved understanding of the magnitude and risk factors of post-discharge mortality is needed to help inform policymakers with the development of risk-guided management policies to reduce paediatric mortality. In this retrospective cohort study, we aimed to evaluate the magnitude and predictors of increased post-discharge mortality among young children admitted with severe anaemia or other syndromes in a high malaria transmission setting in western Kenya.

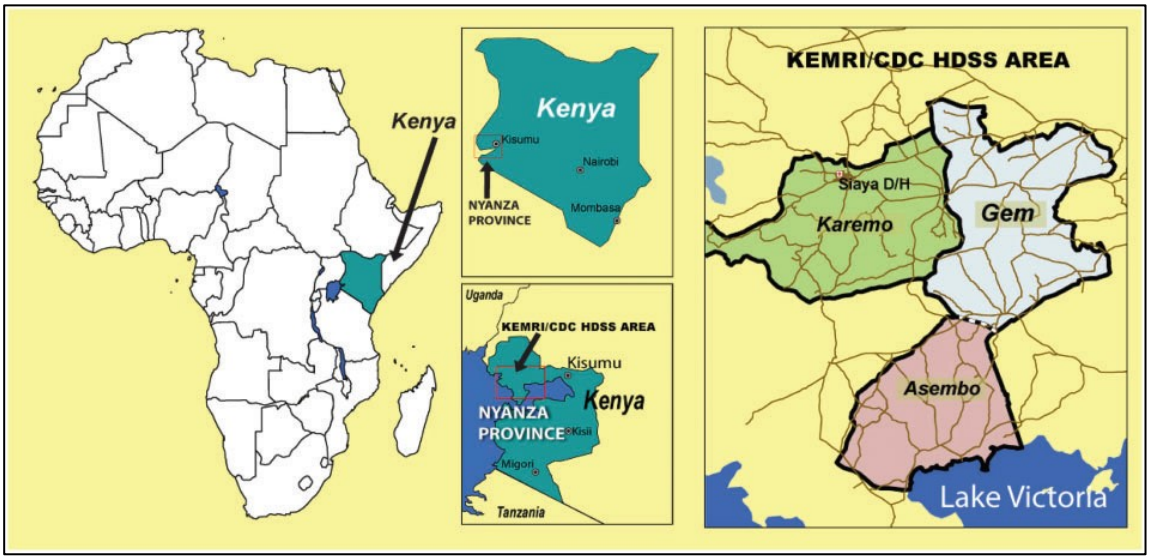
Methods

Data sources and study setting

This study used two sources of data including data from the Kenya Medical Research Institute (KEMRI) and Centers for Disease Control and Prevention (CDC) Health and Demographic Surveillance System (HDSS) platform in Siaya County, Western Kenya (Figure 1)[14] and continuous paediatric in-hospital surveillance in Siaya County referral hospital (formerly Siaya District Hospital). The HDSS provided population-based demographic information collected by trained community health workers through 4-monthly rounds of home visitations comprising of information on births, deaths, morbidity, pregnancies and delivery outcomes, and migration. The in-hospital surveillance provided data on all paediatric admissions up to 12 years of age. The hospital and demographic

surveillance data were linked using the HDSS unique personal identification numbers and fingerprinting[14] to monitor deaths occurring after hospital discharge.

Figure 1: Map of KEMRI/CDC Health and Demographic Surveillance System in western Kenya



Adapted from Odhiambo et al.[14]

Participants

This retrospective cohort analysis included all children aged <5 years admitted to the paediatric department of Siaya County Referral Hospital from 2008-2013 inclusive who were residing within the HDSS study area.[14] Admissions due to surgery, trauma/injury, malignancy or sickle cell anaemia were excluded.

Exposure groups

Each hospital record was examined and categorised based on the diagnosis on discharge or in-hospital death, and the admission syndrome was used where these records were missing. The hospital admissions were categorised in the following primary admission syndromes: severe anaemia (defined as haemoglobin <5.0 g/dL or requiring a blood transfusion), severe malaria (defined as microscopy or rapid diagnostic test confirmed malaria infection and receipt of parenteral treatment with artesunate or quinine but in the absence of severe anaemia), severe pneumonia, and severe acute malnutrition (SAM). Diagnosis of pneumonia and SAM were as given by the routine hospital staff based on the Kenya Ministry of Health guidelines for clinical management during the study period[15] as follows: Severe pneumonia for infants <2months of age was defined as fever (axillary temperature ≥38^{0}C), fast breathing (≥60 breaths per minute [bpm]), grunting, severe chest wall indrawing, refusal to feed and difficulty to wake. For children >2 months

to 5 years, severe pneumonia was defined as: cough, fast breathing (≥50 and ≥40 bpm for 2-12 months and ≥12 months to 5 years respectively), lower chest wall indrawing, stridor, reduced conscious level. SAM was defined as a weight-for-height z-score <-3 SD or a mid-upper-arm-circumference (MUAC) of <11.0 cm, with or without oedema. Children who did not have any of these four diagnoses were pooled under `other syndromes` and children admitted with conditions comprising more than one of the above diagnoses were categorised under `combination syndromes` for subgroups analyses.

Data analysis

The primary outcome was all-cause death. The follow-up time for the in-hospital mortality analysis was defined as the number of days between the date of admission and either the date of in-hospital death or the date of discharge +1. For analysis of the post-discharge mortality, this was defined as the number of days + 1 between the date following the date of discharge and either the date of death or the date of censoring. All children were censored if they left the study because of out-migration, reached five years of age, date of death, or if the study ended before the event had occurred (December 31, 2013).

Categorical variables were described as proportions (percentages), and continuous variables were described as means and corresponding standard deviations (SD) or as median with corresponding interquartile ranges (IQR). Univariate and multivariable Cox regression analyses were used for the analysis of time to the first event. Factors associated with mortality in the univariate analysis ($p<0.2$) were included in the multivariable models as covariates. These included: gender, age at admission, bed net use, antibiotics use, blood transfusion, mother's education status, mother's HIV status, socio-economic status, haemoglobin (Hb) levels on admission and distance from the hospital. There was Insufficient data for the child's HIV status to allow the inclusion of this variable in any of the analyses. Hazard ratios (HR) and their 95% confidence intervals (CI) were reported with two-sided p-values <0.05 regarded as statistically significant.

The Mantel-Haenszel odds ratio (MHOR) for paired binary outcomes was used to compare in-hospital and post-discharge mortality rates.[16] Forest plots were used for the graphical presentation of hazard ratios and Kaplan-Meier plots for the visualisation of the time to first event data.

Data were analysed using STATA version 14 (College Station, Texas, USA).

Ethical review

This study is nested under the HDSS protocol which was approved by the ethical review boards of the Kenya Medical Research Institute (SSC # 647) and the US Centers of Disease Control and Prevention (IRB # 3308). Informed written consent was obtained from compound heads for the participation of their families in all aspects of the HDSS.[14]

Results

Characteristics of the study groups

Between January 1, 2008, and December 31, 2013, a total of 69,492 children living in the combined HDSS and hospital catchment areas met the inclusion criteria. During this period, there were 3,639 hospital admissions involving 4,423 different diagnoses. The primary diagnosis included: severe anaemia (n=655), severe malaria (n=1033), pneumonia (n=996), SAM (n=271) and 'other syndromes' (n=1,468). The median (IQR) follow-up time was 23.6 (10.3-36.0) months and the mean (SD) age on admission was 17.8 (13.7) months (range 13.1-22.2). Over half (59.5%) of children used a bednet. These characteristics differed between primary exposure syndromes (Table 1), including the median age at admission and the median duration of follow-up. Because the duration of follow-up varied between syndromes, the maximum duration was truncated at 12 months. Overall, 4.6% and 10.5% of children were lost to follow-up by three- and six-months post-discharge, respectively.

In-hospital mortality

The overall in-hospital crude mortality rates (MR) was 2.8% (101 of 3,639 admissions) with a median (IQR) length of stay in hospital of 3 (2-5) days. Children admitted with SAM had the highest mortality (MR=9.2%) and the longest median (IQR) stay in hospital of 7 (5-11) days. Severely anaemic children had the second-highest mortality (MR=5.9%) and the median (IQR) length of stay in hospital was 3 (2-5) days. The in-hospital MR among children admitted with pneumonia was 2.7%, and this was 1.8% for `other syndromes` and 1.0% for severe malaria. Multi-variate models comparing the risk of in-hospital mortality in children with and without certain syndromes suggested that the in-hospital mortality was significantly higher in children admitted with severe anaemia compared to those admitted for other reasons (i.e. all other children admitted without severe anaemia, but with pneumonia, or severe malaria or SAM, etc) (hazard ratio [HR]=2.64, 1.58-4.41, p<0.001). The in-hospital mortality was also higher among children admitted with SAM compared to children without SAM (HR=1.84, 1.11-3.05, p=0.018) but similar among children with

and without pneumonia (HR=0.86, 0.52-1.44, p=0.575) or 'other syndromes' (HR=0.69, 0.37-1.30, p=0.253). By contrast, the risk of dying in-hospital was lower among children with severe malaria vs those without (HR=0.38, 0.22-0.67, p=0.001) (Table 2)

Post-discharge mortality

The cumulative post-discharge mortality among the 3,538 survivors was 7.6%, 9.4%, and 12.4% by three, six- and twelve-months post-discharge, respectively (Table 2). By six months, all-cause post-discharge mortality was highest in the SAM group (MR=37.3%), followed by severe anaemia (MR=24.1%), pneumonia (MR=8.3%), 'other syndromes' (MR=7.9%), and then severe malaria (MR=1.0%) (Table 2). Multi-variate models comparing the risk of post-discharge mortality in children with and without certain syndromes suggested the post-discharge mortality by six months was significantly higher in children with SAM vs without SAM (HR=3.86, 2.56-5.81, p<0.001) and with vs without severe anaemia group (HR=2.46, 1.69-3.56, p<0.001). By contrast, the 6-month post-discharge mortality was similar among children with and without pneumonia (HR=0.89, 0.60-1.33, p=0.578) and with and without 'other syndromes' (HR=0.98, 0.61-1.56, p=0.917). It was lower in children admitted with severe malaria vs those without severe malaria (HR=0.31, 0.20-0.49, p<0.001) (Table 2).

Because similar trends were seen for both in-hospital and post-discharge mortality (Figure 2), the cumulative all-cause mortality that combined the in-hospital with post-discharge mortality by 6 months also showed a similar ranking by syndrome: SAM (34/71, 47.9%), severe anaemia (48/152, 31.6%), (45/365, 12.3%), `other syndromes' (99/943, 10.5%) and severe malaria (8/316, 2.5%) (Table 2)

Table 1: Baseline characteristics of study participants by the primary diagnosis on admission

Characteristic	Severe anaemia	Severe malaria	Pneumonia	Malnutrition	Other syndromes	Combination syndromes	Total
Syndrome on index admission—no./total no.	255/3639	499/3639	586/3639	130/3639	1468/3639	701/3639	3639/3639
Sex (male)—no./total no. (%)	130/255 (51.0%)	256/499 (51.3%)	302/586 (51.5%)	64/130 (49.2%)	800/1468 (54.5%)	374/701 (53.4%)	1926/3639 (52.9%)
First admission age (months)—mean±SD	22±14.4	22.2±14.8	13.1±11.3	16.3±10.2	18.9±14.3	15.0±12.0	17.8±13.7
Bed net use—no./total no. (%)	97/255 (38.0%)	432/499 (86.6%)	366/586 (62.5%)	82/130 (63.1%)	636/1468 (43.3%)	552/701 (78.7%)	2165/3639 (59.5%)
Distance—median (IQR)	7.4 (4.1-10.5)	5.5 (2.3-9.5)	7.3 (2.9-9.8)	7.8 (3.5-10.9)	6.4 (2.3-10.1)	7.4 (4.2-10.3)	6.8 (2.9-10.0)
Discharge during malaria season—no./total no.	112/255 (43.9%)	240/499 (48.1%)	289/586 (49.3%)	64/130 (49.2%)	714/1468 (48.6%)	343/701 (48.9%)	1762/3639 (48.4%)
SES—median (IQR)	-0.2 (-0.6-0.3)	0.3 (-0.4-0.3)	-0.1 (-0.5-0.3)	-0.3 (-0.6-0.1)	-0.1 (-0.4-0.3)	-0.2 (-0.5-0.2)	-0.1 (-0.4-0.3)
Mother survival status—no./total no. (%)							
Alive	244/255 (95.7%)	483/499 (96.8%)	574/586 (98.0%)	126/130 (96.9%)	1413/1468 (96.3%)	687/701 (98.0%)	3527/3639 (96.9%)
Death	2/255 (0.8%)	1/499 (0.2%)	4/586 (0.7%)	1/130 (0.8%)	12/1468 (0.8%)	3/701 (0.4%)	23/3639 (0.6%)
Unknown	9/255 (3.5%)	15/499 (3.0%)	8/586 (1.4%)	3/130 (2.3%)	43/1468 (2.9%)	11/701 (1.6%)	89/3639 (2.4%)
Farther survival status—no./total no. (%)							
Alive	227/255 (89.0%)	432/499 (86.6%)	507/586 (86.5%)	117/130 (90.0%)	1229/1468 (83.7%)	580/701 (82.7%)	3092/3639 (85.0%)
Death	8/255 (3.1%)	16/499 (3.2%)	27/586 (4.6%)	6/130 (4.6%)	66/1468 (4.5%)	34/701 (4.9%)	157/3639 (4.3%)
Unknown	20/255 (7.8%)	51/499 (10.2%)	52/586 (8.9%)	7/130 (5.4%)	173/1468 (11.8%)	87/701 (12.4%)	390/3639 (10.7%)
Mother HIV status—no./total no. (%)							
Positive	27/255 (10.6%)	40/499 (8.0%)	83/586 (14.2%)	23/130 (17.7%)	173/1468 (11.8%)	96/701 (13.7%)	442/3639 (12.1%)
Negative	109/255 (42.7%)	251/499 (50.3%)	256/586 (43.7%)	43/130 (33.1%)	628/1468 (42.8%)	338/701 (48.2%)	1625/3639 (44.7%)
Unknown	119/255 (46.7%)	208/499 (41.7%)	247/586 (42.2%)	64/130 (49.2%)	667/1468 (45.4%)	267/701 (38.1%)	1572/3639 (43.2%)
Mother Education level—no./total no. (%)							
Lower primary	10/255 (3.9%)	10/499 (2.0%)	22/586 (3.8%)	8/130 (6.2%)	51/1468 (3.5%)	27/701 (3.9%)	128/3639 (3.5%)
Upper primary	152/255 (59.6%)	286/499 (57.3%)	334/586 (57.0%)	68/130 (52.3%)	785/1468 (53.5%)	423/701 (60.3%)	2048/3639 (56.3%)

Table 1: Baseline characteristics of study participants by the primary diagnosis on admission

Characteristic	Severe anaemia	Severe malaria	Pneumonia	Malnutrition	Other syndromes	Combination syndromes	Total
Secondary	85/255 (33.3%)	174/499 (34.9%)	202/586 (34.5%)	46/130 (35.4%)	528/1468 (36.0%)	226/701 (32.2%)	1261/3639 (34.7%)
Tertiary	6/255 (2.4%)	21/499 (4.2%)	25/586 (4.3%)	5/130 (3.8%)	83/1468 (5.7%)	13/701 (1.9%)	153/3639 (4.2%)

SES, socioeconomic status; IRQ, interquartile range; `Other syndromes` denote the undefined syndromes in children that did not have severe anaemia, severe malaria, pneumonia or severe acute malnutrition; `combination syndromes` comprise children with a combination of more than one syndrome including severe anaemia, severe malaria, pneumonia malnutrition or `other syndromes`

Table 2: outcomes by the primary diagnosis on admission

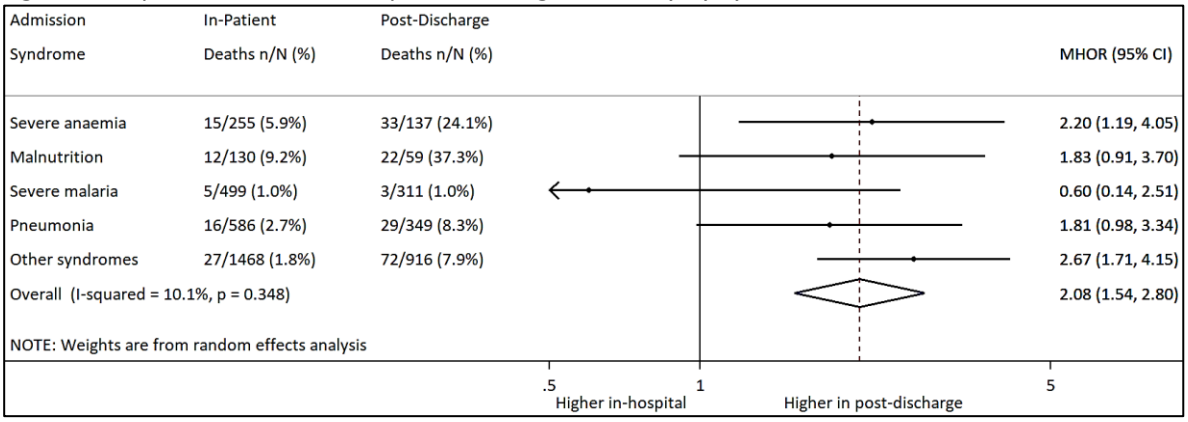
Outcomes	Severe anaemia	Severe malaria	Pneumonia	Malnutrition	`Other syndromes`	Total
Duration of hospital stay during initial admission in days— median (IQR)	3 (2-5)	3 (2-5)	4 (3-6)	7 (5-11)	3 (2-5)	3 (2-5)
Duration of follow-up (months)	22.3 (5.9-35.7)	23 (11.3-34.1)	24.4 (10.2-35.7)	15.7 (3-31)	25.2 (11.1-38.7)	23.6 (10.3-36)
Loss to follow-up by:						
3 months	10/255 (3.9%)	35/499 (7.0%)	18/586 (3.1%)	8/130 (6.2%)	65/1468 (4.4%)	169/3639 (4.6%)
6 months	27/255 (10.6%)	61/499 (12.2%)	56/586 (9.6%)	16/130 (12.3%)	147/1468 (10.0%)	382/3639 (10.5%)
12 months	47/255 (18.4%)	122/499 (24.4%)	111/586 (18.9%)	25/130 (19.2%)	290/1468 (19.8%)	728/3639 (20.0%)
Deaths						
In-hospital	15/255 (5.9%)	5/499 (1.0%)	16/586 (2.7%)	12/130 (9.2%)	27/1468 (1.8%)	101/3639 (2.8%)
Post-discharge deaths (total follow-up time)	43/240 (17.9%)	16/494 (3.2%)	59/570 (10.4%)	28/118 (23.7%)	116/1441 (8.0%)	335/3639 (9.2%)
Total in-hospital and post-discharge deaths	58/240 (24.2%)	21/494 (4.3%)	75/570 (13.2%)	40/118 (33.9%)	143/1441 (9.9%)	436/3639 (12%)
Median (IQR) time to post-discharge death (months)	1.8 (0.3-4.1)	7.5 (4.8-8.1)	1.8 (0.4-6.4)	0.6 (0.2-2)	1.1 (0.4-4)	2.1 (0.6-7.1)
Risk of death from discharge to:						
3 months	25/137 (18.2%)	0/311 (0.0%)	26/349 (7.4%)	21/59 (33.9%)	62/916 (6.8%)	167/2173 (7.6%)

Table 2: outcomes by the primary diagnosis on admission

Outcomes	Severe anaemia	Severe malaria	Pneumonia	Malnutrition	`Other syndromes`	Total
6 months	33/137 (24.1%)	3/311 (1.0%)	29/349 (8.3%)	22/59 (37.3%)	72/916 (7.9%)	205/2173 (9.4%)
12 months	40/137 (29.2%)	11/311 (3.5%)	45/349 (12.9%)	23/59 (39.0%)	92/916 (10.0%)	269/2173 (12.4%)
Cumulative risk for in-hospital and post-discharge deaths up to:						
3 months	40/152 (26.3%)	5/316 (1.6%)	42/365 (11.5%)	33/71 (46.5%)	89/943 (9.4%)	268/2274 (11.8%)
6 months	48/152 (31.6%)	8/316 (2.5%)	45/365 (12.3%)	34/71 (47.9%)	99/943 (10.5%)	306/2274 (13.5%)
12 months	55/152 (36.2%)	15/316 (4.7%)	61/365 (16.7%)	35/71 (49.3%)	119/943 (12.6%)	370/2274 (16.3%)
Hazard ratio for in-hospital mortality	2.64 (1.58-4.41), p<0.001	0.38 (0.22-0.67), p=0.001	0.86 (0.52-1.44), p=0.575	1.84 (1.11-3.05), p=0.018	0.69 (0.37-1.3), p=0.253	
Hazard ratio for post-discharge mortality						
3 months	2.38 (1.57-3.59) p<0.001	0.25 (0.14-0.42) p<0.001	0.79 (0.52-1.20) p=0.264	3.94 (2.54-6.11) p<0.001	0.900 (0.55-1.49) p=0.691	
6 months	2.46 (1.69-3.56) p<0.001	0.31 (0.20-0.49) p<0.001	0.89 (0.60-1.33) p=0.578	3.86 (2.56-5.81) p<0.001	0.98 (0.61-1.56) p=0.917	
12 months	2.23 (1.59-3.12) p<0.001	0.39 (0.27-0.57) p<0.001	0.82 (0.57-1.17) p=0.264	2.79 (1.88-4.13) p<0.001	0.82 (0.54-1.25) p=0.349	

'Other syndromes` denote the undefined syndromes in children that did not have severe anaemia, severe malaria, pneumonia or severe acute malnutrition.
IQR, interquartile range.
Post-discharge mortality risk (follow-up truncated to 12 months).

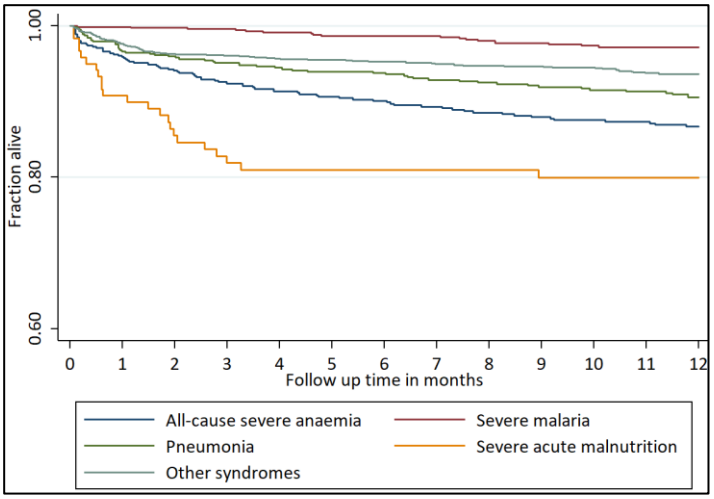
Figure 2: In-patient vs six-month post-discharge mortality by syndrome



MHOR, Mantel-Haenszel odds ratio; CI, confidence interval; `Other syndromes` denote the undefined syndromes in children that did not have severe anaemia, severe malaria, pneumonia or severe acute malnutrition.

The Kaplan-Meier plot (Figure 3) illustrates that. among the post-discharge deaths by 12 months (n=269/2173, 12.4%), the median (IQR) time to death was 2.1 (0.6-7.1) months overall (i.e. 50% of all death by 12 months occurred within the first 2.1 months), with 62.1% (167/269) and 76.2% (205/269) of these deaths occurring within the first three and six months respectively. The median time to death was shortest, and the proportion of the death that had occurred by three months was highest, for children with SAM (0.6m, 21/23 [91.3%]) followed by `other syndromes` (1.1m, 62/92 [67.4%]), severe anaemia (1.8m, 25/40 [62.5%]), pneumonia (1.8m, 26/45 [57.8%]) and severe malaria (7.5m, 0/11 [0%]).

Figure 3: Survival curves showing time to post-discharge death



`Other syndromes` denote the undefined syndromes excluding severe anaemia, severe malaria, pneumonia or severe acute malnutrition.

Overlapping syndromes

The co-presence of SAM was associated with significantly higher 6-month post-discharge mortality in children with severe anaemia compared to severely anaemic children that did not have SAM (MR=25.1% vs MR=10.0%, HR=2.49, 1.18-5.26, p=0.017) (Figure 4). The co-existence of SAM also significantly increased the 6-month post-discharge mortality in children with pneumonia (MR=14.8% vs MR=5.0%, HR=2.91, 1.41-6.01, p=0.004) and children with severe malaria (MR=12.2% vs 2.3%, HR=5.29, 2.11-13.24, p<0.001). The co-existence of severe anaemia did not significantly increase the 6-month mortality in SAM children (MR=25.1% vs 21.2%, HR=1.22, 0.56-2.64, p=0.613), but increased it significantly in those with severe malaria (MR=6.6% vs 1.4%, HR=4.67, 2.07-10.58, p<0.001). The co-existence of severe malaria was associated with lower 6-month post-discharge mortality in children with severe anaemia; i.e. children with severe malarial anaemia had a lower risk of dying post-discharge than children with severe non-malarial anaemia (MR=6.6% vs 14.8%, HR=0.45, 0.25-0.80, p=0.007) (Figure 4).

In-hospital vs post-discharge mortality

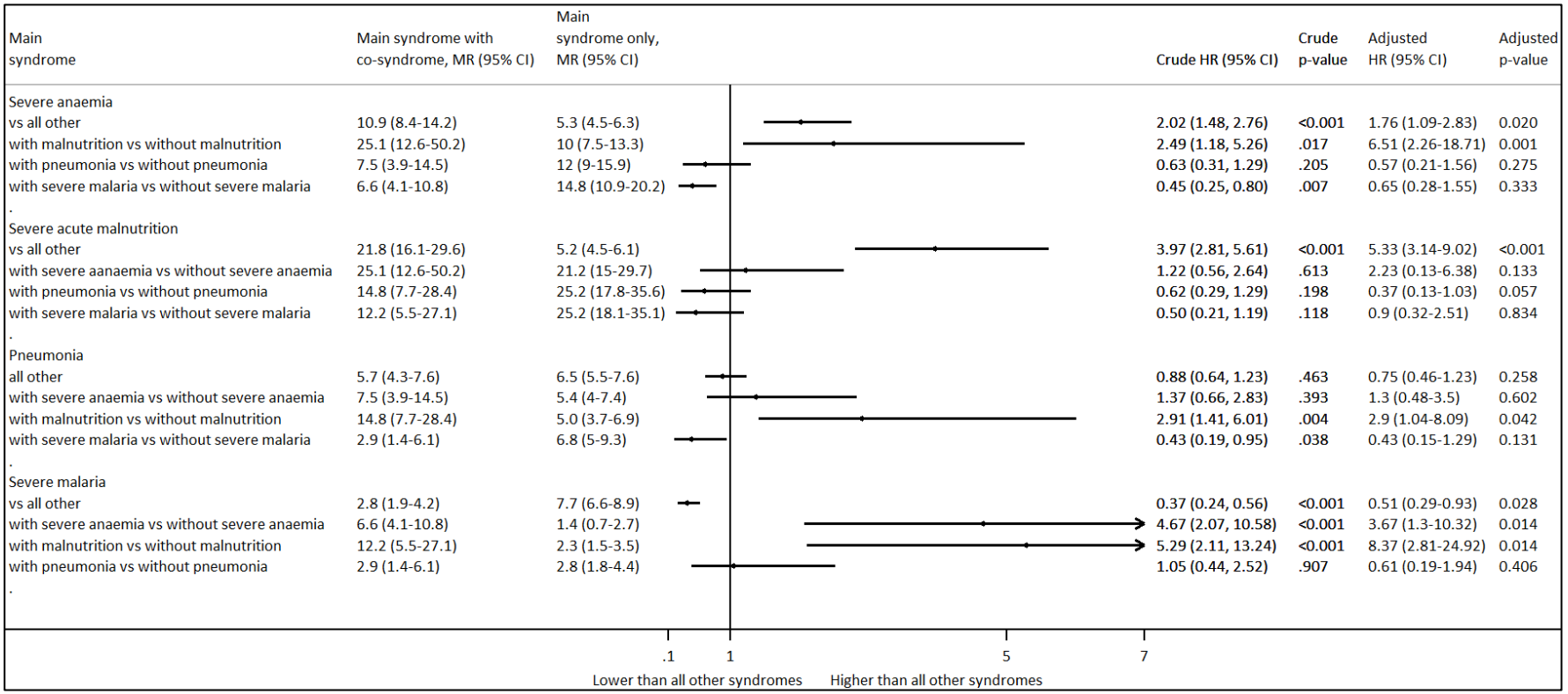
Overall, the odds of dying in the first six months post-discharge was higher than during the in-hospital period (MHOR=2.08, 1.54-2.80, p<0.001) with relatively little variation between syndromes. The differences were statistically significant for severe anaemia, and `other syndromes` (Figure 2).

Other risk factors for post-discharge mortality

In the univariate model, post-discharge mortality by six months decreased with increasing age (Table 3) and Figure S1) from MR=9.5% in infants aged <6 months to MR=7.0% in the 6 to <18 months old (HR=0.74, 0.53-1.02, p=0.067) and MR=4.1% in the 36 to <60 months old group (HR=0.38, 0.21-0.67, p<0.001). HIV-exposed children were twice as likely to die than HIV-unexposed children during the 6-month follow-up period (HR=2.61, 1.68-4.07, p<0.001). The HIV status was only available for 5.5% of the children, so no analysis by child's HIV status was conducted. Post-discharge mortality decreased with increasing socioeconomic status (SES) from MR=10.1% in the poorest terciles to MR=7.4% in the middle tercile (HR-0.79, 0.57-1.08, p=0.134) to MR=5.4% in the highest tercile (HR=0.51, 0.35-0.73, p<0.001). Maternal education was also associated with decreasing post-discharge mortality, but this was only significant for secondary level education (MR=5.3%) compared to lower primary education (MR=9.9%, HR=0.50, 0.27-0.92, p=0.027). Increasing distance from the admitting hospital was associated with higher post-discharge mortality with children with the tercile that lived furthest having a more than 2-fold higher hazard compared

to those in the first tercile (HR=2.66, 1.82-3.87, p<0.001). Bed net use was associated with a significant reduction in the six months post-discharge mortality (HR=0.65, 0.49-0.85, p=0.002). Treatment with antibiotics during in-patient care was not associated with post-discharge mortality (HR=1.10, 0.82-1.48, p=0.507). Among children with severe anaemia, lack of receipt of blood transfusion was not associated with a statistically significantly increased post-discharge mortality (HR=1.86, 0.24-14.35, p=0.553). However, a unit increase in Hb levels on admission (prior to receipt of any blood transfusion) was associated with about 46% decrease in the risk of 6 months post-discharge mortality (HR=0.54, 0.38-0.77, p<0.001). Gender and discharge during malaria season were not associated with post-discharge mortality.

Figure 4: Six months post-discharge mortality by main syndrome relative to 'co-syndromes`



Main syndrome	Main syndrome with co-syndrome, MR (95% CI)	Main syndrome only, MR (95% CI)	Crude HR (95% CI)	Crude p-value	Adjusted HR (95% CI)	Adjusted p-value
Severe anaemia						
vs all other	10.9 (8.4-14.2)	5.3 (4.5-6.3)	2.02 (1.48, 2.76)	<0.001	1.76 (1.09-2.83)	0.020
with malnutrition vs without malnutrition	25.1 (12.6-50.2)	10 (7.5-13.3)	2.49 (1.18, 5.26)	.017	6.51 (2.26-18.71)	0.001
with pneumonia vs without pneumonia	7.5 (3.9-14.5)	12 (9-15.9)	0.63 (0.31, 1.29)	.205	0.57 (0.21-1.56)	0.275
with severe malaria vs without severe malaria	6.6 (4.1-10.8)	14.8 (10.9-20.2)	0.45 (0.25, 0.80)	.007	0.65 (0.28-1.55)	0.333
Severe acute malnutrition						
vs all other	21.8 (16.1-29.6)	5.2 (4.5-6.1)	3.97 (2.81, 5.61)	<0.001	5.33 (3.14-9.02)	<0.001
with severe aanaemia vs without severe anaemia	25.1 (12.6-50.2)	21.2 (15-29.7)	1.22 (0.56, 2.64)	.613	2.23 (0.13-6.38)	0.133
with pneumonia vs without pneumonia	14.8 (7.7-28.4)	25.2 (17.8-35.6)	0.62 (0.29, 1.29)	.198	0.37 (0.13-1.03)	0.057
with severe malaria vs without severe malaria	12.2 (5.5-27.1)	25.2 (18.1-35.1)	0.50 (0.21, 1.19)	.118	0.9 (0.32-2.51)	0.834
Pneumonia						
all other	5.7 (4.3-7.6)	6.5 (5.5-7.6)	0.88 (0.64, 1.23)	.463	0.75 (0.46-1.23)	0.258
with severe anaemia vs without severe anaemia	7.5 (3.9-14.5)	5.4 (4-7.4)	1.37 (0.66, 2.83)	.393	1.3 (0.48-3.5)	0.602
with malnutrition vs without malnutrition	14.8 (7.7-28.4)	5.0 (3.7-6.9)	2.91 (1.41, 6.01)	.004	2.9 (1.04-8.09)	0.042
with severe malaria vs without severe malaria	2.9 (1.4-6.1)	6.8 (5-9.3)	0.43 (0.19, 0.95)	.038	0.43 (0.15-1.29)	0.131
Severe malaria						
vs all other	2.8 (1.9-4.2)	7.7 (6.6-8.9)	0.37 (0.24, 0.56)	<0.001	0.51 (0.29-0.93)	0.028
with severe anaemia vs without severe anaemia	6.6 (4.1-10.8)	1.4 (0.7-2.7)	4.67 (2.07, 10.58)	<0.001	3.67 (1.3-10.32)	0.014
with malnutrition vs without malnutrition	12.2 (5.5-27.1)	2.3 (1.5-3.5)	5.29 (2.11, 13.24)	<0.001	8.37 (2.81-24.92)	0.014
with pneumonia vs without pneumonia	2.9 (1.4-6.1)	2.8 (1.8-4.4)	1.05 (0.44, 2.52)	.907	0.61 (0.19-1.94)	0.406

HR, hazard ratio; CI, confidence interval; MR, mortality rate; Other syndromes` not included, there were no deaths for overlapping syndromes between `other syndromes` and severe anaemia or severe malaria. Adjusted estimates were obtained from multivariate models that included the following covariates: age at admission, bed net use, socio-economic status, distance to hospital, maternal education level and mother's HIV status.

Table 3: Risk factors for 6-month post-discharge mortality

	Univariate HR (95%CI)	P	Multivariate HR (95%CI)	P
Admission age (ref: <6 months)				
6 to <18 months	0.74 (0.53-1.02)	0.067	0.63 (0.39-1.09)	0.100
18 to <36 months	0.42 (0.28-0.64)	<0.001	0.40 (0.21-0.79)	0.008
36 to <60 months	0.38 (0.21-0.67)	0.001	0.52 (0.22-1.22)	0.134
Gender (ref: female)	1.07 (0.81-1.41)	0.632		
Haemoglobin (mean [SD])	0.54 (0.38-0.77)	<0.001	1.06 (0.97-1.15)	0.222
Bed net (ref: no use)	0.65 (0.49-0.85)	0.002	0.76 (0.47-1.22)	0.259
Discharge season (ref: non-malaria season)	0.96 (0.49-1.26)	0.760		
Blood transfusion (ref: not transfused)	1.86 (0.24-14.35)	0.553		
Antibiotics	1.10 (0.82-1.48)	0.507		
Socioeconomic status (ref: first tercile-poorest)				
Second tercile	0.79 (0.57-1.08)	0.134	1.08 (0.66-1.75)	0.758
Third tercile	0.51 (0.35-0.73)	<0.001	0.53 (0.29-0.96)	0.037
Distance from the hospital (ref: first tercile, closest)				
Second tercile	1.86 (1.25-2.78)	0.002	1.50 (0.79-2.82)	0.214
Third tercile	2.66 (1.82-3.87)	<0.001	2.00 (1.07-3.74)	0.029
Mother education (ref: lower primary)				
Upper primary	0.62 (0.34-1.11)	0.108	0.48 (0.23-1.00)	0.051
Secondary	0.50 (0.27-0.92)	0.027	0.40 (0.17-0.91)	0.028
Tertiary	0.53 (0.22-1.30)	0.166	0.62 (0.15-2.58)	0.511
Mother HIV status (ref: HIV negative)	2.61 (1.68-4.07)	<0.001	2.06 (1.24-3.40)	0.005

HR, hazard ratio; HIV, human immunodeficiency virus; Hb, haemoglobin; The multivariable model adjusts for age category, Hb levels on admission bed net use, SES terciles, distance terciles, mother`s education status and mother's HIV positive status.

Discussion

In this malaria-endemic area in western Kenya, children with severe anaemia and SAM had both the highest in-hospital and post-discharge mortality, followed by children admitted with pneumonia. Except for severe malaria, children were at two-fold higher risk of dying within six months after discharge than during the initial hospital period. Among children admitted with severe anaemia half of these post-discharge deaths by 12 months occurred in the first 1.8 months and this was 0.6 months for children with SAM, which was similar in children admitted with pneumonia, where the median survival time was 1.8 months, but which was associated with lower post-discharge mortality overall than SAM or severe anaemia. Overall, factors associated with post-discharge mortality were comparable with earlier reports from similar epidemiological settings including; young age, HIV-exposure, lower maternal education, distance from the hospital and haemoglobin levels on admission.[10,17] Unfortunately, the HIV-status of the child was not available for most of these children.

Our findings are consistent with previous observational studies in the same study area in Kenya,[11] Malawi[10] and Uganda[9] which also reported high rates of post-discharge mortality among children admitted with all-cause severe anaemia. In our study, 18.2% had died by three months and 24.1% by six months while in the earlier observational study in Kenya, 18.8% died over two months[11] and in Malawi, 8.8% died in six months.[10] Of note is that our observed post-discharge mortality rates are considerably higher than those previously reported in the placebo arms of three post-discharge malaria chemoprevention trials in children with severe anaemia in Malawi[18,19] and Kenya (Chapter 6). It is likely that the children enrolled in these trials benefitted from access to better in-hospital care and closer follow-up post-discharge than can be achieved in routine settings.

Overall, half of the post-discharge deaths by 12 months, occurred in the first 2.1 months (the median time to death). This pattern differed between syndromes (Figure 3) as in children with SAM, nearly all of the deaths had occurred by three months post-discharge, and this was approximately 62% for children with severe anaemia and 57% for children with pneumonia. By contrast, for children with severe malaria, the time to death was spread over the year with a median survival time of 7.5 months. The first few months post-discharge has been recognised as a period of high vulnerability to mortality and morbidity in children with SAM and severe anaemia, possibly because of physiological stress and impairment of the immune system.[17,20,21] In malaria-endemic areas Plasmodium falciparum infection has been shown to lead to endothelial inflammation which may

persist for more than a month even after the parasites have been cleared.[22] This is likely to predispose to or worsen untreated bacterial infections,[23] including invasive non-typhoidal salmonella infections (iNTS) which have been shown to have a positive correlation with Plasmodium falciparum infection in malaria-endemic areas in sub-Sahara Africa.[24] Children with coexisting iNTS and falciparum malaria infections have a high in-hospital mortality rate[25] which is even worse in children with severe malarial anaemia,[26] and likely contributes to the high post-discharge mortality in the immediate post-discharge period.

The high risk of post-discharge mortality in children with SAM and severe anaemia was also evident in our analysis of the impact of co-existence of multiple syndromes. Overall, we found that children with pneumonia and severe malaria had the worst outcomes if they also had SAM or severe anaemia. For example, the 6-month post-discharge mortality was about five-fold higher in children with severe malaria if they also had SAM or severe anaemia. Similarly, children with severe pneumonia were at a threefold higher risk if they also had SAM. By contrast, the co-existence of pneumonia or malaria did not increase the post-discharge mortality in children with SAM or severe anaemia.

Due to insufficient data, we could not explore the causes of post-discharge death, and these are likely to be multifactorial in this setting, including HIV infection, other acute and chronic infections, e.g. tuberculosis, and malnutrition or micronutrient deficiencies. However, previous studies in similar epidemiological settings among children admitted with severe anaemia have reported malaria as the main reason for morbidity and mortality in the post-discharge period. In Uganda,[9] 89% of re-hospitalisations and 59% of clinic visits were due to malaria, and in previous studies in this same study area in western Kenya, 30% of post-discharge deaths were due to malaria.[11] In a similar setting in Kenya, 50% of participants were parasitaemic one-month post-discharge.[12] Full recovery from malaria-associated anaemia is known to take up to 6 weeks,[27] possibly due to continued dyserythropoietic and bone marrow suppression[28,29] and new infections or recrudescence due to poor parasite clearance are likely to cause further delays in recovery.

Another noticeable finding was the relatively low risk of post-discharge mortality in children with severe malaria in the absence of SAM or severe anaemia. Severe malaria is an acute condition of which proper diagnosis and treatment result in better prognosis as opposed to the multifactorial aetiology of non-malaria anaemia in this setting which is difficult to diagnose and treat, especially due to inadequate human and structural capacity in the healthcare system.[30] In this setting, malaria and anaemia are diagnosed clinically following the Integrated management of childhood illnesses

(IMCI) guidelines.[31] Parenteral antimalaria treatment and blood transfusion are then administered presumptively, mostly without further investigation for other possible underlying conditions. Therefore, inadequate care during in-hospital stay due to challenges in diagnosis and treatment may result in disease progression and poor post-discharge prognosis among children with non-malaria severe anaemia. Another possible reason is the misclassification of uncomplicated malaria as severe malaria, therefore underestimating the severe malaria-specific mortality rates; it has been shown that about a third of admissions for 'severe malaria' do not fulfil WHO's criteria for severe malaria in malaria-endemic sub-Sahara Africa.[32]

Almost all post-discharge deaths in this study (>95%) occurred in the community before children were referred or able to reach the hospital. These findings are consistent with a recent study in a HDSS area in Mozambique where > 80% of post-discharge deaths in children was reported to have occurred outside the hospital[17] and in another study among children admitted with infectious conditions in Uganda where 67% of post-discharge deaths occurred in the community or on the way to the hospital.[33] Delay in seeking care due to ignorance of the danger signs of severe anaemia and preference for traditional medicine are likely contributors to high post-discharge mortality among children with severe anaemia in Uganda.[34] We found lower maternal education to be positively associated with post-discharge mortality. Thus, an intervention, including maternal/caretaker education on discharge on how to identify the danger signs and to seek prompt care, may help reduce the burden. This is further supported by the above-mentioned finding that the post-discharge mortality rates in the placebo arm of the PMC trial conducted in this area (chapter 6) were much lower than in this observational study, similar to the differences in mortality rates between trials and observational cohort studies in Malawi.[10,18]

A major limitation of this study was the lack of data on the HIV status of participants. At the time of the study, testing for HIV among the paediatric population in Kenyan public hospitals was not offered routinely. Although the information on the maternal HIV status was available for many (71.4%), data on the actual HIV status of the child was available in only 5.5%. The study area is known to have a high prevalence of HIV infection,[35] and paediatric HIV infection is a major risk factor for severe anaemia.[36] Studies in areas with high HIV and malaria co-infection rates in Uganda and Kenya have reported up to 5-fold higher 6-month post-discharge mortality rates in children with severe malarial anaemia associated with HIV infection.[36,37] Another major limitation is the lack of additional information about other co-morbidities associated with the primary diagnosis of severe anaemia. The aetiology of severe anaemia in this setting has been shown to be complex and

multifactorial including nutritional deficiencies, hookworm infestations, acute and chronic infections such as malaria, bacteraemia, tuberculosis and Human Immunodeficiency Virus (HIV) infection, genetic disorders such as sickle cell anaemia etc.[1] There was also limited information on diarrhoea and bacteraemia which are generally known to cause substantial morbidity in sub-Sahara Africa. The latter reflects the limited diagnostic capacity in Kenyan public hospitals. Many of the children with sepsis may have ended up in the `other syndromes` group in our analysis.

The results of this study add to the limited evidence from other studies in malaria-endemic areas and confirm that children under five years of age admitted with SAM and severe anaemia are at a high risk of post-discharge mortality in the first few months after discharge. Guidelines exist for the management of children with SAM post-discharge, but similar guidelines need to be developed for the post-discharge management and follow-up of children with severe anaemia. Furthermore, studies of predictors and the aetiology of post-discharge mortality are urgently needed to develop targeted management guidelines for post-discharge care.

Article Information

Contributors

TKK and FOtK conceived the idea. TKK wrote the protocol with input from FOtK and SN. TKK and EO conducted the data abstraction from the HDSS database and cleaned the data. TKK conducted the data analysis with assistance from FOtK. SN provided statistical support. TK and FOtK wrote the first draft of the manuscript. All authors reviewed, revised and approved the final version of the manuscript.

Declaration of interests

There are no conflicts of interest to declare.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

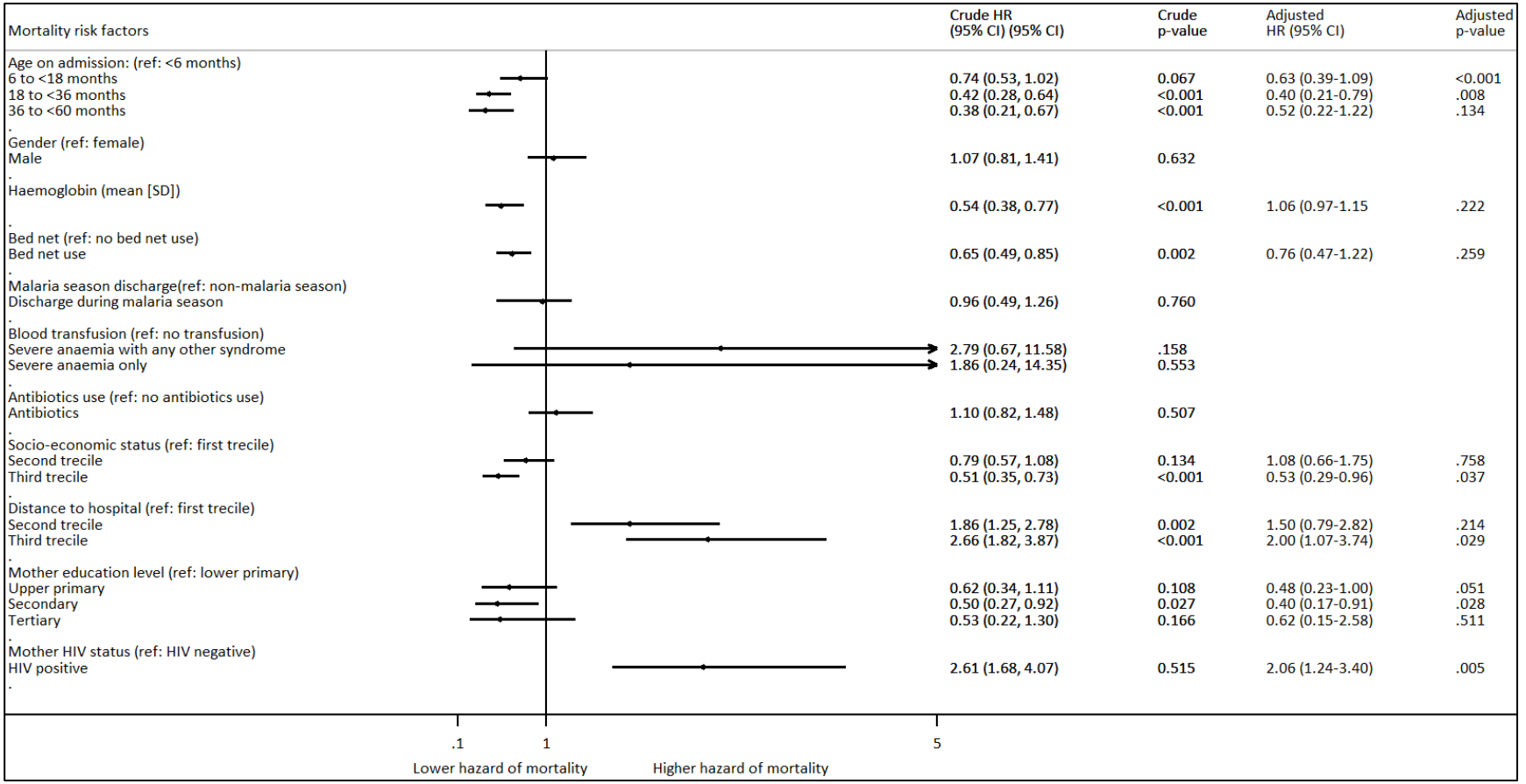
Supplement to:

Titus K Kwambai, Meghna Desai, Menno Smit, Sarah Nevitt, Eric Onyango, Simon Kariuki, Martina Oneko, Aaron Samuels, Mary Hamel, Feiko O ter Kuile.

The Post-Discharge Risk of Mortality in Children Under Five Years of Age Living in A High Malaria Transmission Area in Western Kenya: A Retrospective Cohort Study

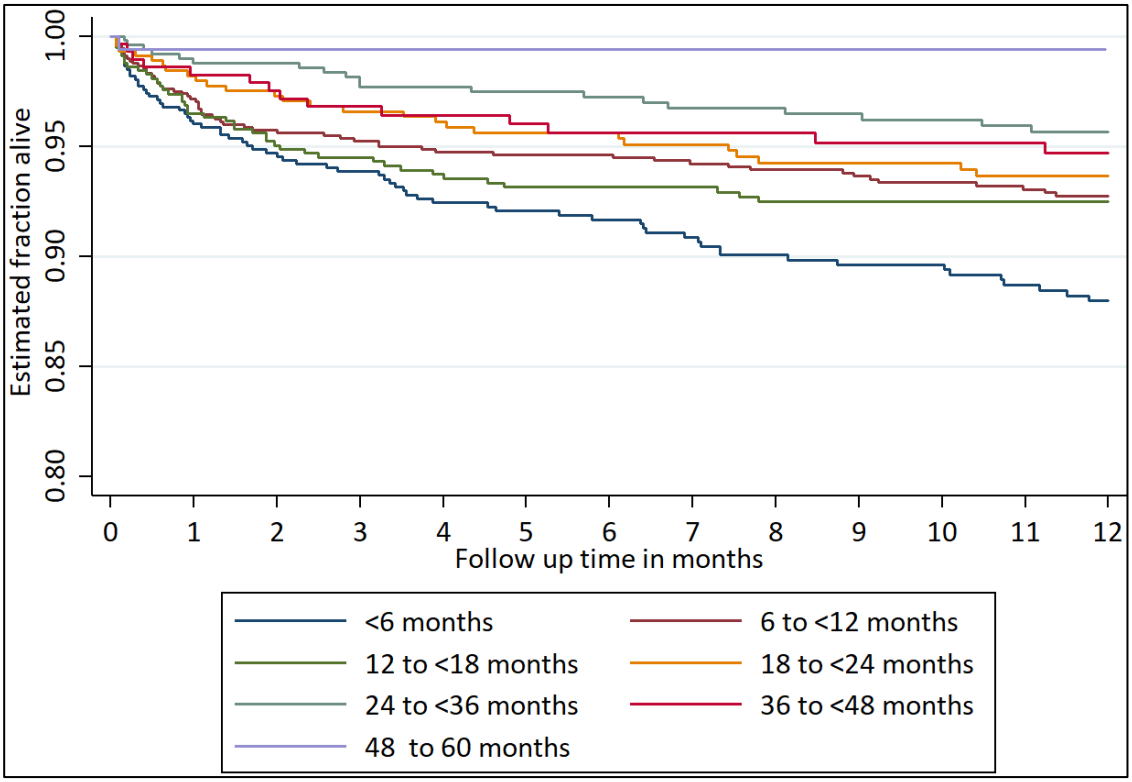
Supplemental figures

Figure S1: Risk factors for 6-month post-discharge mortality



HR, hazard ratio; CI, confidence interval

Figure S2: Kaplan Meier curve showing post-discharge mortality by age category



Chapter 4: Post-Discharge Morbidity and Mortality Systematic Review and Meta-Analysis

Post-Discharge Risks of Morbidity and Mortality in Children Admitted with Severe Anaemia and Other Syndromes in Malaria-Endemic Settings in Africa: A Systematic Review and Meta-Analysis

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Abstract

Introduction

Severe anaemia is associated with high in-hospital mortality among young children. Several studies in malaria-endemic areas have shown that surviving children remain at increased risk of dying or re-admission for several months after discharge from hospital.

Methodology

We conducted a systematic review and meta-analysis to determine the pooled risks of morbidity and mortality in the post-discharge period among children admitted with severe anaemia versus other syndromes in malaria-endemic areas in Africa. Two authors independently searched medical databases up to October 31, 2018, for prospective and retrospective cohort studies and clinical trials involving children aged <15 years. The primary outcomes were all-cause death and readmission within six months post-discharge. We used random-effect meta-analyses.

Results

Eighteen studies fulfilled the entry criteria and were included. The duration of post-discharge follow-up varied from 28 days to 1 year. The odds of dying within 6 months post-discharge was higher than during the in-hospital period for children admitted with severe anaemia (N=4, Mantel-Haenszel odds ratio =1.44, 95% CI 1.07-1.92, p<0.0157, I^2=0.8%) and they were twice as likely to die by 6 months post-discharge compared with children admitted without severe anaemia (N=4, Relative Risk [RR]=2.80, 1.61-4.86, p<0.0001, I^2=73.1%). Severe malnutrition was also associated with increased post-discharge mortality compared to children admitted without severe anaemia or malnutrition (N=2, RR=4.27, 2.42-7.55, p<0.0001, I^2=76.8). The risk of readmission was higher among children admitted with severe anaemia compared to other syndromes, but this was based on only one study (RR=3.05, 1.12-8.35, p<0.001, I^2=0.0%).

Conclusion

In malaria-endemic areas of Africa, children hospitalised with severe anaemia and severe malnutrition are at an increased risk of dying in the first six months post-discharge compared to children admitted with other syndromes. There is a need to develop post-discharge management strategies for this high-risk group.

Introduction

Substantial progress has been made in the past decade in reducing child mortality globally, but in sub-Saharan Africa, about 1 in 13 children still die before their fifth birthday.[1] Severe anaemia and malaria are major contributors to morbidity and mortality in malaria-endemic areas of Africa.[2,3] Severe anaemia alone accounts for 2-29% of all paediatric hospitalisations[4-6] and about a third of children hospitalised with fever in Africa also have severe anaemia.[7] The mortality due to severe anaemia among children during the in-hospital period ranges from 4 to 10% in different epidemiological settings in Africa.[8-10] The aetiology in these settings is complex and multifactorial and include nutritional causes,[11,12] and acute and chronic infections such as malaria, tuberculosis, human immunodeficiency virus (HIV) infections, bacteraemia and hookworm infestations.[13]

Recent studies among children with severe anaemia have focused on interventions to reduce high in-hospital mortality.[14-16] However, it is now increasingly recognized that the excess risk of mortality continues even after discharge from hospital[17-20] with up to 33% of the children dying or being readmitted during the first six months post-discharge.[8,21] The post-discharge period is a well-recognised risk period for children with severe acute malnutrition,[22,23] but is less studied in children with severe anaemia.

We hypothesised that children admitted with severe anaemia constitute an especially vulnerable group of post-discharge mortality and morbidity due to a combination of environmental, behavioural, nutritional and genetic risk factors.[13,19] We conducted a systematic review and meta-analysis to compare the pooled risks of post-discharge mortality and re-admissions among children admitted with all-cause severe anaemia versus other syndromes without severe anaemia in malaria-endemic areas of Africa. We also compared the in-hospital mortality against post-discharge mortality.

Methods

Search strategy and selection criteria

This analysis was completed per the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement.[24] The protocol was registered in PROSPERO: The International Prospective Register of Systematic Reviews (CRD42017079282).

Search strategy

We identified eligible studies by performing a systematic and comprehensive electronic literature search using a combination of search terms (Supplement 1) in PubMed, SCOPUS, EMBASE, Web of Science and Cochrane CENTRAL from inception to October 31, 2018, without language restrictions. We identified other relevant studies by scanning reference lists of all identified articles and searching in Google and Google Scholar.

Eligibility criteria

Prospective or retrospective cohort studies and randomised-controlled trials (RCTs) were eligible for inclusion if they: (i) presented original data with or without comparator groups (ii) included children <15 years of age admitted with severe anaemia or other syndromes such as malaria, pneumonia, diarrhoea, malnutrition, HIV etc., alone or in combination with severe anaemia; (iii) defined the duration of the post-discharge follow-up and (iv) were conducted in malaria-endemic countries in Africa according to the World Malaria Report 2017.[25]

Studies or sub-groups were excluded if they (i) involved admissions for sickle cell anaemia, malignancies or surgery, road accidents and other trauma cases (ii) did not report follow-up data for in-patients separate from out-patient.

Study selection and data extraction

Two independent reviewers (TKK and ATM) screened titles, abstracts and full texts of the identified articles and agreed on the final eligibility. Any disagreement between the two reviewers was resolved through consensus or after consultation with a third reviewer (FOtK).

TKK and ATM independently extracted the data using a standardised form and database. If required, additional information was obtained from the authors. For each study, the following information was extracted: first author's name, year of publication, recruitment site, study design, geographic location, study period, age range of participants, comparison groups, main syndrome on admission, number of enrolled participants, inpatient mortality, post-discharge mortality, post-discharge morbidity, post-discharge follow-up time, eligibility criteria, outcomes and reported risk estimates. In trials or other comparative studies where interventions were provided, only the data from the control groups that received the standard of care were included.

Quality assessment

The Cochrane Collaboration's tool was used to assess the quality and risk of bias of clinical trials.[26] The quality of observational studies and cohort studies with comparison groups were assessed using the Newcastle Ottawa Scale.[27] For cohort studies without comparison groups, a modified version of the Newcastle Ottawa Scale was used that omitted the comparability criteria and in the "selection criteria" the section on "selection of the non-exposed cohort".

Data analysis

Two primary outcome measures were used: all-cause death or all-cause re-admissions in the post-discharge period.

Data were analysed using STATA version 14.0 (Stata Corporation, College Station TX). DerSimonian and Laird random-effects meta-analysis were used to generate pooled relative risks (RR). Pooled effect estimates using fixed-effects models (Mantel-Haenszel) were also provided as part of sensitivity analysis and are only provided in the forest plots.

The Mantel-Haenszel odds ratio (MHOR) for paired binary outcomes was used to compare in-hospital and post-discharge mortality rates.[28]

Heterogeneity was expressed using the I^2 statistic and categorised as low, moderate, substantial and considerable if the I^2 value ranged between 0-40%, 30-60%, 50-90%, and 75-100%, respectively.[29]. We used two-tailed p-values of <0.05 to indicate statistical significance. We intended to perform sensitivity analysis to determine the influence of study quality on results, but this was not possible due to the small numbers of studies included in the meta-analysis and lack of heterogeneity in study quality.

Role of the funding source

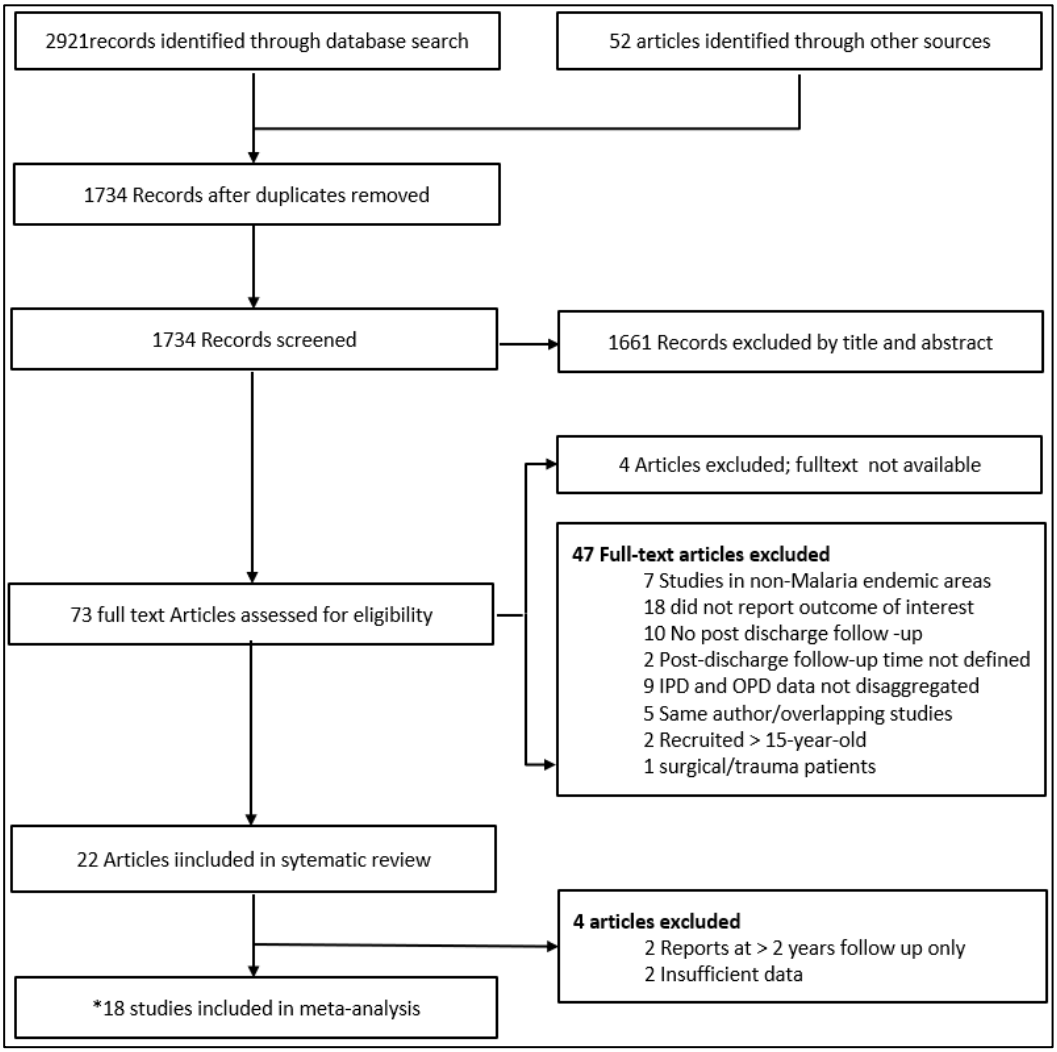
The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Our search identified 2,972 articles; after removal of duplicates and screening of titles and abstracts, 73 full-text articles were further evaluated, of which 18 were eligible; this included 16 cohort studies[17,19,20,23,30-41] and 2 RCTs[42,43] (Figure 1 and Table 1). The studies were published

between 1987 and 2018 and were conducted in Kenya (five), Malawi (four), Uganda (two), The Gambia (one), Guinea-Bissau (two), Democratic Republic of Congo (two) and Tanzania (two). The main syndromes on admission included severe anaemia (six studies), malaria (three), pneumonia (four), malnutrition (three) and five other studies in which the main syndromes on admission were not specified (unspecified syndromes). The post-discharge follow-up period ranged from 28 days to 5 years but was truncated to a maximum of 12 months for analysis. The post-discharge mortality ranged from 1 to 39% (Table 1). The Cochrane Collaboration tool for RCTs scored the two RCTs as low risk of bias (eTable 1). Fifteen cohort studies were scored as good quality and one as poor quality (eTable 2, eTable 3).

Figure 1: PRISMA Diagram



IPD, in-hospital; OPD, outpatient department. Reasons for exclusion exceed the number of articles excluded because some articles have more than one reason for exclusion.
*including a prospective follow-up study of children who were initially enrolled in a prospective case-control study.[20]

Table 1: Characteristics of included studies

Study	Location	Dates	Comparison groups	Age Range	Admission Syndrome	Total Enrolled	IP Death	PD Death	Re-Admission	FUP Time
Randomised Controlled Trials										
Biai et al.[42]	Guinea-Bissau	2004-06	Control ward	3m-5y	Malaria	491	9.6%	1%	NR	28d
Phiri et al.[43]	Malawi	2006-09	Placebo	4m-59m	SMA	708	NA	2.3%	18.5%	6m
Cohort studies without a comparator group										
Carme et al.[30]	Congo	1988-89	All enrolled	≤ 14	Cerebral malaria	170	15%	6.7%	NR	27m
Chhibber et al.[32]	The Gambia	2008-12	All enrolled	2m-59m	Pneumonia+ others	3735	3.9%	2.8%	NR	180d
Villamor et al.[39]	Tanzania	1993-97	All enrolled	6m-60m	Pneumonia	687	3.1%	11.7%	NR	Mean
Wiens et al.[40]	Uganda	2012-13	All enrolled	6m-5y	All-cause (Infectious)	1307	5.0%	4.9%		24.7m
Hennart et al.[34]	Congo	1970	All enrolled	mean 46m	PEM	171	NR	0.18	NR	5y
Kerac et al.[23]	Malawi	2006-07	All enrolled	5m-14y	PEM	1024	23.2%	24.0%	7.1%	1y
Cohort studies with comparator groups										
Moisi et al.[35]	Kenya	2003-08	Post-discharge group	<15y	All-cause	14971	NR	4.5%	8.9%	12m
			Community group	<15y	NR					
Ngari et al.[36]	Kenya	2007-12	Post-discharge group	1m-59m	Severe Pneumonia	2461	5.6%	3.1%	NR	1y
			Post-discharge group	1m-59m	No Severe Pneumonia	5270	2.4%	1.3%	NR	
Opoka et al.[19]	Uganda	2008-13	Admissions with cerebral malaria	18m-12y	Cerebral malaria	162	12.7%	0.6%	3.1%	6m
			Admissions with SMA	18m-12y	SMA	138	0.4%	2.2%	9.4%	
			Community (not admitted)	18m-12y	Healthy	133	NA	0%	0%	

Table 1: Characteristics of included studies

Study	Location	Dates	Comparison groups	Age Range	Admission Syndrome	Total Enrolled	IP Death	PD Death	Re-Admission	FUP Time
Veirum et al.[38]	Guinea-Bissau	1991-96	PD Cohort	≤15y	All-cause	2950	12.5%	7.5%	15.9%	12m
			Community cohort	≤15y	All-cause	8184	NA	*MRR	0.041	
Zucker et al.[41]	Kenya	1991	Exposed group	6m-5y	Severe anemia	293	13.0%	18.8%	NR	8w
			non-exposed group	6m-5y	Non- Severe anemia	930	8.9%	11.3%	NR	
Snow et al.[37]	Kenya	1992-97	Post-discharge group	≤6y	All-cause	1148	0.03	2.39/1000 pm	347	1y
			Community group	≤6y	NR	2845	NA	1.06/1000 pm	172	
Phiri et al.[20]	Malawi	2002-06	Cases	6-60m	Severe anemia	377	6.4%	11.6%	17.2%	18m
			Hospital control	6-60m	No severe anemia	377	0.0%	2.7%	9.4%	
			Community controls	6-60m	Healthy	380	N/A	1.3%	10.0%	
Kwambai et al.[17]	Kenya	2008-2013	Admitted with severe malaria	≤5y	Severe malaria	499	1.0%	3.5%	NR	12m
			Admitted with severe anaemia	≤5y	Severe anaemia	255	5.9%	29.2%	NR	
			Admitted with pneumonia	≤5y	Pneumonia	586	2.7%	12.9%	NR	
			Admitted with malnutrition	≤5y	Malnutrition	130	9.2%	39.0%	NR	
			Admitted with Other syndromes	≤5y	Other syndromes	1468	1.8%	10.0%	NR	
Chinkhumba et al.[33]	Malawi	2005-06	HIV Positive	6m-59m	SAM	79	30.4%	7.3%	NR	4m
			HIV Negative	6m-59m	SAM	375	8.5%	2.0%	NR	
Hau et al.[31]	Tanzania	2014	All-enrolled	2-12Y	All-cause	537	7.7%	12.6	NR	12m

Table 1: Characteristics of included studies

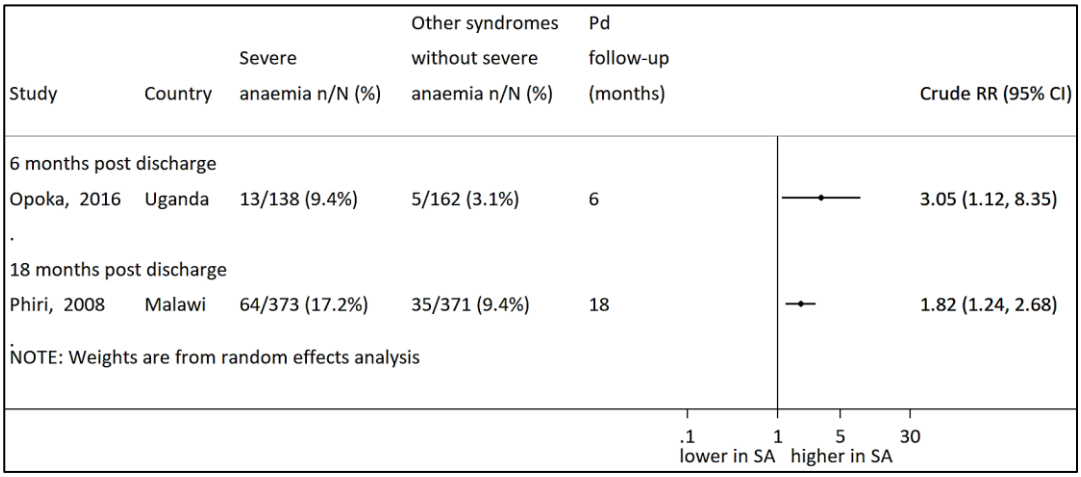
Study	Location	Dates	Comparison groups	Age Range	Admission Syndrome	Total Enrolled	IP Death	PD Death	Re-Admission	FUP Time

IP, inpatient; PD, post-discharge; FUP, follow-up period; SP, sulphadoxine-pyrimethamine; IPTpd, intermittent preventive treatment post-discharge; SpO2, peripheral capillary oxygen saturation; NR, not reported; SMA, severe malarial anaemia; SAM, severe acute malnutrition; NA, not applicable; MRR, mortality rate ratio; PEM, protein-energy malnutrition.

Post-discharge readmission

Seven studies reported post-discharge readmissions for periods ranging between 3 to 18 months with wide variations in the reported readmission rates (Table 1). The crude proportion of children re-admitted at least once by six months was 11.6% (201/1740) ranging from 3.1% to 18.5% based on three studies.[19,23,43] Readmissions for severe anaemia and non-anaemia by six months post-discharge was 17.0% and 6.4% respectively based on these three studies. Only two studies allowed for a direct comparison of re-admission risk by syndrome,[19,20] but the results were not pooled because they reported readmission rates at different times; 6 and 18 months respectively (Figure 2). There were no studies to conduct a comparative meta-analysis for readmission rates for other syndromes.

Figure 2: Post-discharge readmission between severe anaemia and other syndromes without severe anaemia.

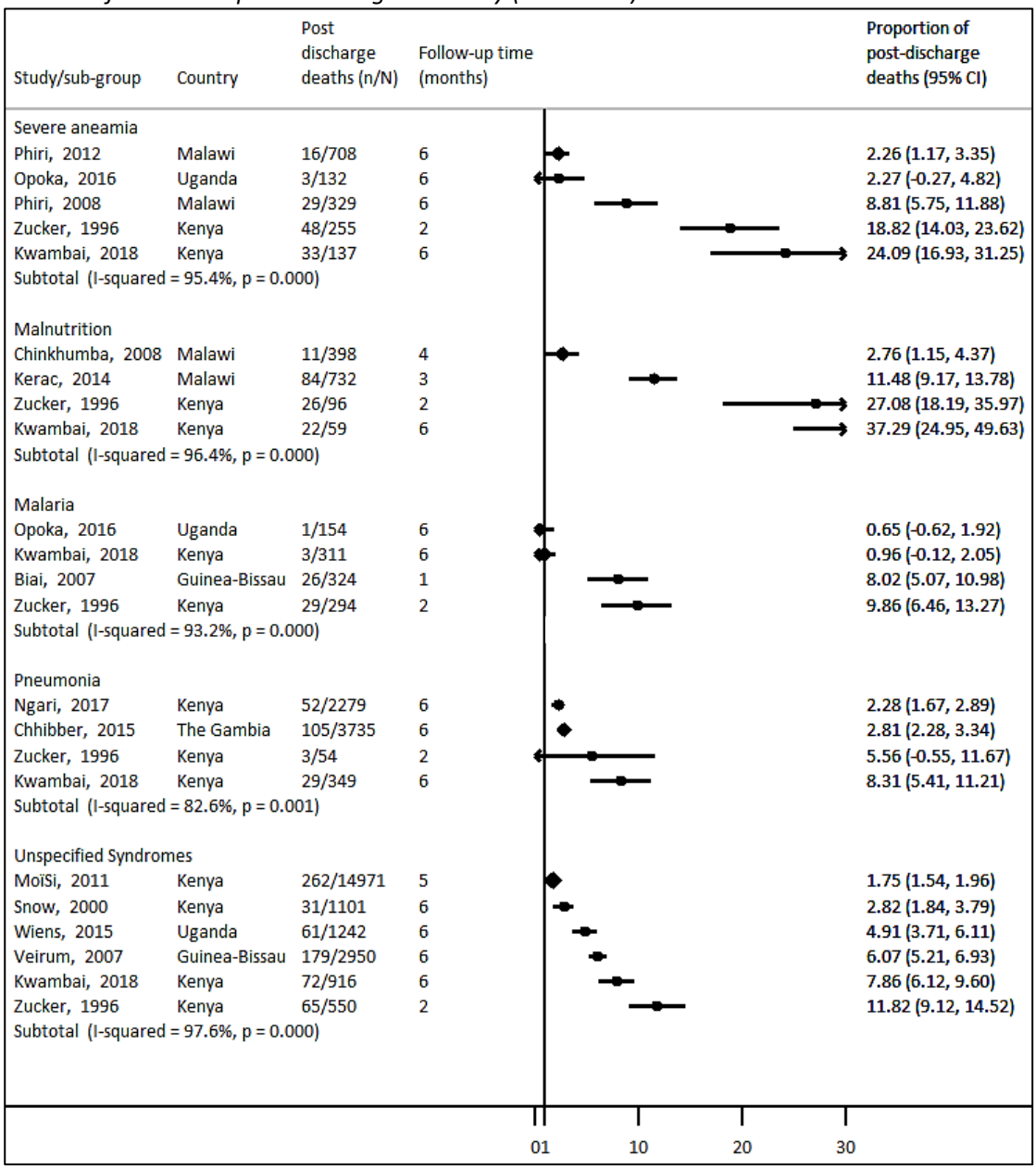


RR, relative risk; SA, severe anaemia; CI, confidence interval; D+L, DerSimonian and Laird; M-H, Mantel-Haenszel.

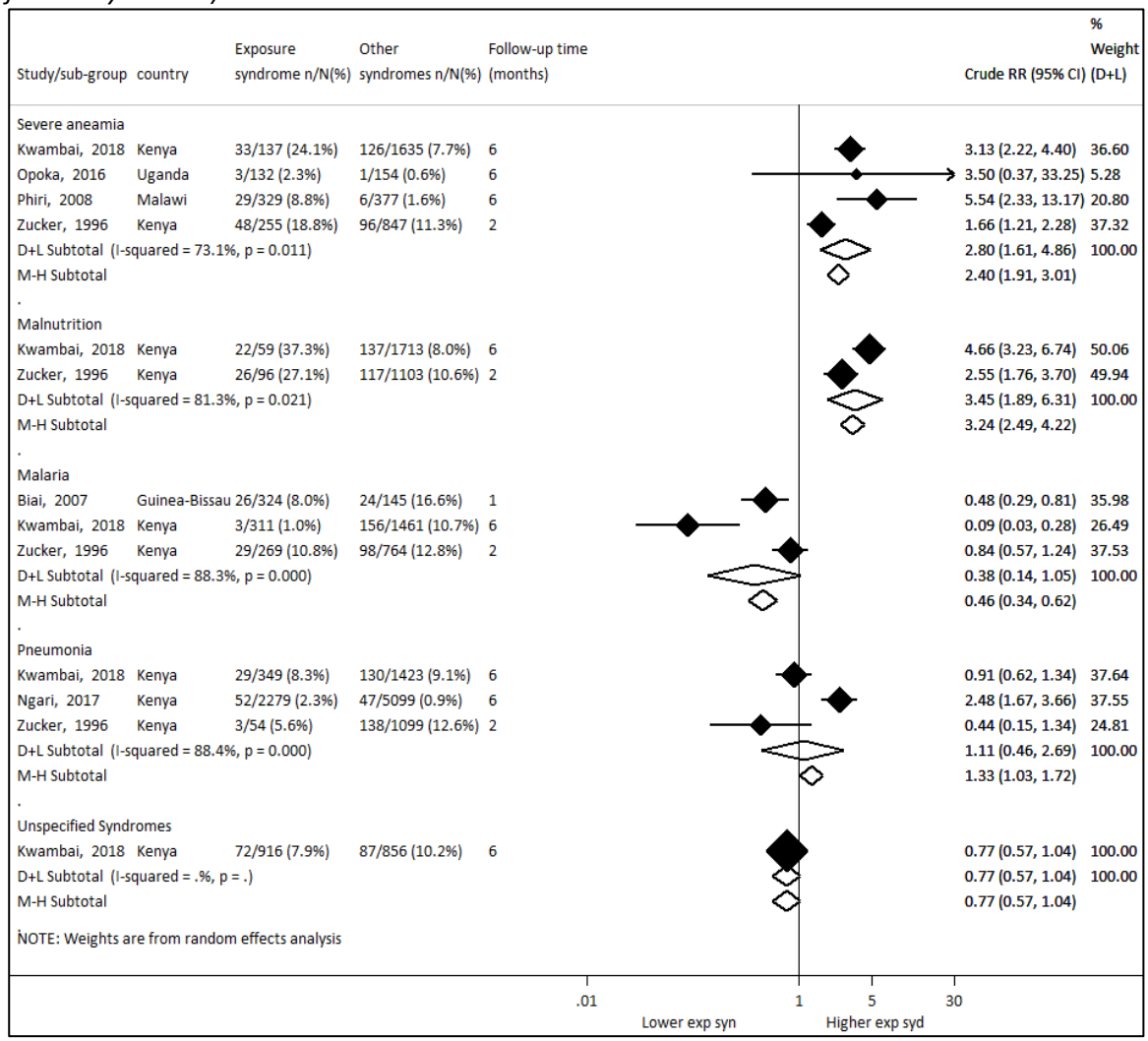
Post-discharge mortality by syndrome

A meta-analysis of the post-discharge mortality from 14 studies that reported this measure by syndrome showed that there was considerable heterogeneity between (I^2=95.6%) and within admission syndrome groups (I^2>80% for all). The crude mortality was 3.6% (1141/32034), but because of the high level of heterogeneity, a pooled summary proportion obtained by meta-analysis was not calculated (Figure 3). The mortality by six months post-discharge ranged from 2.3%-24.1%, 2.8%-37.3%, 0.7%-9.9%, 2.3%-8.3% and 1.8%-11.8% for severe anaemia, malnutrition, malaria, pneumonia, and unspecified syndromes respectively. The 12-month post-discharge mortality by syndrome (17 studies) is shown in eFigure 1.

Figure 3: Mortality by six months post-discharge
3a. Risk of six-month post-discharge mortality (all studies).



3b. Relative Risk of six-month post-discharge mortality (excludes studies that only reported results for one syndrome).



RR, relative risk; CI, confidence interval; D+L, DerSimonian and Laird; M-H, Mantel-Haenszel.
Top panel a: Kwambai 2018, Zucker 1996 and Opoka, 2016 are included in more than one sub-group each representing a mutually exclusive group. The lower CI for Opoka, 2016 and Kwambai, 2018 and Zucker, 1996 is negative due to the small number of post-discharge deaths reported, the lower CIs should be treated as zero. Due to considerable heterogeneity (as shown by the I^2) between and within admission syndrome groups the summary statistics are not shown. The pooled I^2 is 95.6%.
Bottom panel b: Includes only studies that reported enough detail to allow direct comparisons of the post-discharge mortality by syndrome among children from the same cohort study

Among the 14 studies that reported mortality by six months, six involved cohort studies that reported enough detail to allow direct comparisons of the post-discharge mortality by syndrome (Figure 3, Table 2). This showed that children admitted with severe anaemia were 2.80 times more likely to die in the first six months post-discharge than children admitted during the same study period and in the same hospitals without severe anaemia (N=4, RR=2.80, 1.61-4.86, p<0.001, I^2=73.1%). Severe malnutrition was also associated with higher post-discharge mortality (N=2, RR=3.45, 1.89-6.31, p<0.001, I^2=81.3%). The post-discharge mortality among children admitted with severe pneumonia, malaria and unspecified

syndromes were similar or lower compared to children admitted for other reasons. Similar trends were observed by 12 months post-discharge (eFigure 1, Table 2).

When the analysis was repeated, but now excluding children (post-hoc) with either severe anaemia, or with severe malnutrition from the comparator group, the relative risk for 6-month post-discharge mortality for children with severe anaemia relative to other children when malnourished children were excluded from the reference group was 2.43 (N=2, 1.07-5.51, p<0.033, I^2=90.5%). There was insufficient data for a similar comparison by twelve months (Table 2). For malnutrition, this was nearly 4-fold higher by six months when compared to a reference that excluded the children with severe anaemia (N=2, RR=4.27, 2.42-7.55, p<0.001, I^2=76.8%). This was 3-fold higher by twelve (N=2, RR=3.70, 2.84-4.82, P<0.001, I^2=0.0%) (Table 2).

Children who had both severe anaemia plus malaria (i.e. severe malarial anaemia) had a lower risk of post-discharge mortality than children with severe anaemia without evidence of malaria (N=2, RR=0.44, 0.02-0.86, p<0.040, I^2=75.9%) (eFigure 2).

Five further studies evaluated HIV status as a risk factor; HIV was associated with a 3-fold increased risk of post-discharge mortality (HR=3.39, 1.58-5.19, p<0.001, I^2=68.2%).[20,23,31,36,39] This was observed in two studies before the widescale introduction of anti-retroviral therapy (ART) (HR=4.64, 0.62-8.66, p=0.24, I^2=16.4%)[20,39] and in three studies after the introduction of ARTs (HR=3.02, 0.59-5.45, p<0.15, I^2=80.6%)[23,31,36] (eFigure 5).

Table 2: Comparison by syndrome of the risks of post-discharge mortality and re-admissions by six and by twelve months post-discharge

	By 6 months			By 12 months		
	RR (95% CI, P, I^2, N) Versus any other syndrome	RR (95% CI, P, I^2, N) Versus any other syndrome, excluding severe anaemia	RR (95% CI, P, I^2, N) Versus any other syndrome, excluding severe malnutrition	RR (95% CI, P, I^2, N) Versus any other syndromes	RR (95% CI, P, I^2, N) Versus any other syndrome, excluding severe anaemia	RR (95% CI, P, I^2, N) Versus any other syndrome, excluding severe malnutrition
Mortality						
Severe anaemia	2.80 (1.61-4.86), P<0.001, 73.1%, 4	NA	2.43 (1.07-5.51), P<0.033, 90.5%, 2	2.49 (1.53-4.06), P<0.001, 73.0%, 4	NA	Insufficient data
Severe malnutrition	3.45 (1.89-6.31), P<0.001, 81.3%, 2	4.27 (2.42-7.55), P<0.001, 76.8%, 2	NA	3.03 (2.18-4.21), P<0.001, 40.6%, 2	3.70 (2.84-4.82), P<0.001, 0.0%, 2	NA
Severe pneumonia	1.11 (0.46-2.69), P=0.821, 88.4%, 3	1.25 (0.56-2.79), P=0.584, 85.1%, 3	0.88 (0.48-1.63), P=0.690, 32.6%, 2	1.64 (0.75-3.61), P=0.214, 91.6%, 2	2.14 (0.82-5.63), P=0.122, 93.6%, 2	1.16 (0.87-1.56), P=0.305, 60.0%, 2
Not defined	1.00 (0.59-1.70), P=0.996, 79.4%, 2	0.98 (0.52-1.85), P=0.951, 85.4%, 2	1.03 (0.84-1.26), P=0.770, 0.0%, 2	0.96 (0.53-1.75), P=0.905, 85.4%, 2	1.10 (0.74-1.63), P=0.646, 64.4%, 2	0.89 (0.60-1.34), P=0.591, 79.4%, 2
Severe malaria	0.38(0.14-1.05), P=0.062, 88.3%, 3	0.34 (0.03-4.10), P=0.393, 94.2%, 2	0.33 (0.003-4.15), P=0.390, 94.3, 2	0.50 (0.24-1.06), P=0.071, 85.0%, 3	0.55 (0.16-1.87), P=0.336, 91.3%, 2	0.53 (0.15-1.89), P=0.328, 91.9%, 2
Re-admissions						
Severe anaemia	3.05 (1.12-8.35), P<0.001, 0.0%, 1	NA	Insufficient data	Insufficient data	N/A	Insufficient data

RR=relative risk, CI confidence interval. The effect estimates shown in the second column from the left is the same as shown in figure 3b (given for illustration purposes only)

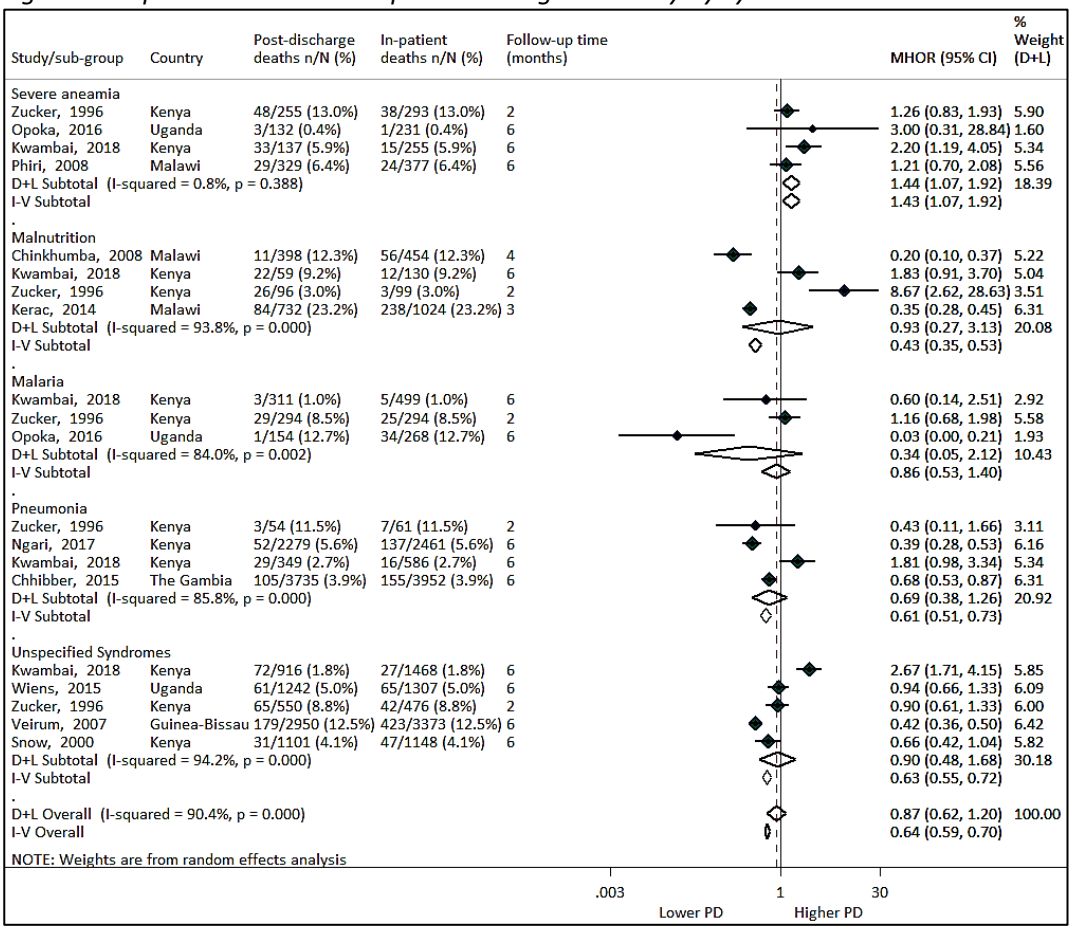
Post-discharge versus in-hospital mortality

During the in-hospital period 7.3% (1501/20574) of children died ranging from 0.4% to 23.2% of all children admitted (N=15), and again a pooled summary proportion obtained by meta-analysis was not calculated because of the high level of heterogeneity between studies (I^2=96.9%) (eFigure 3). The respective mortality by syndrome ranged between 0.4%-13% for severe anaemia and 3.0%-23.2%, 1.0%-15.1%, 2.7%-11.5% and 1.8%-12.5% for malnutrition, malaria, pneumonia, and unspecified syndromes respectively (eFigure 3). Eleven cohort studies involving a total of 18,474 admissions reported both in-hospital and post-discharge mortality. Overall, across all syndromes pooled, the mortality post-discharge by six months was lower than in hospital (MHOR=0.87, 0.62-1.20, p=0.388, I^2=90.4%). However, there was considerable heterogeneity within and between syndrome sub-groups. Among children admitted with severe anaemia, the odds of mortality were consistently higher in the post-discharge period (N=4, MHOR=1.44, 1.07-1.92, p<0.015, I^2=0.8%) (Figure 4), whereas for all other syndromes this tended to be higher during the in-hospital period (all other syndromes pooled: N=16, MHOR=0.72, 0.49-1.06, p<0.099, I^2=91.4%); malnutrition: MHOR=0.93, 0.27-3.13, p=0.901, I^2=93.8%; malaria: MHOR=0.34, 0.05-2.12, p=0.247, I^2=84.0%; pneumonia: MHOR=0.69, 0.38-1.26, p=0.224, I^2=85.8%; unspecified syndromes: MHOR=0.90, 0.48-1.68, p=0.742, I^2=94.2 (p-value for difference between the effect estimates of severe anaemia versus all other subgroups combined p=0.003) (Figure 4).

Post-discharge mortality vs mortality in the community

Six prospective follow-up studies showed that relative to otherwise healthy children from the community, hospital admission due to any reason was associated with a 3-fold increased mortality rate over six months (N=6, RR=3.32, 1.83-4.82, p<0.001, I^2=17.6%) (eFigure 4). The relative risk was 4.26 (N=2, -32.23-40.74, p=0.819, I^2=0.0%) for children admitted with severe anaemia, 4.30 (N=1, 1.57-7.03, p=0.002) for pneumonia (only 1 study) and 4.02 (N=3, 0.88-7.16, p<0.001, I^2=56.0%) for unspecified syndromes.

Figure 4: In-patient vs six-month post-discharge mortality by syndrome on admission.



PD, post-discharge deaths; D+L, DerSimonian and Laird; MHOR, Mantel-Haenszel odds ratio.

Other risk factors for post-discharge mortality

Nine studies analysed other potential risk factors for post-discharge mortality (eTable 4). Risk factors significantly associated with post-discharge mortality when pooled in a meta-analysis included: discharge against medical advice (HR=6.68, 2.93-10.43), hypoxia (SPO_2<90%) (HR=2.26, 1.51-3.01) and bacteraemia (HR=1.83, 1.18-2.49) (eFigure 5). Other factors significantly associated with post-discharge mortality but could not be pooled by meta-analyses due to differences in definitions and scales used included younger age, previous hospitalisation, mother HIV positive status, poor anthropometric measurements, reduced conscious levels, proteinuria and admission with a chronic disease. One unit increase in oxygen (HR=0.95, 0.92-0.98) and haemoglobin (Hb) levels on admission (HR=0.75, 0.63-0.87) were associated with a reduction in post-discharge mortality. The presence of malaria parasites on admission was also associated with lower post-discharge mortality (HR=0.47, 0.33-0.61). Gender and axillary temperature were not associated with post-discharge mortality (eFigure 5).

Discussion

To our knowledge, this is the first systematic review and meta-analysis that compares the risk of post-discharge mortality in malaria-endemic Africa by syndrome on admission. The review shows that children with severe anaemia and malnutrition were approximately two and three times more likely to die during the first six months post-discharge compared to children admitted with other syndromes. Children with severe anaemia were also more likely to die in the first six months post-discharge than during their in-hospital stay as opposed to children admitted with malnutrition or other syndromes where the mortality tended to be similar or highest during the in-hospital period. Studies with longer follow-up periods showed that most of these events occurred in the first six months following hospital discharge.[19,20] We also found that children originally admitted with severe anaemia were about three times more likely to be readmitted for any cause by six months post-discharge compared to children admitted with other syndromes, but this was based on only one study. These findings indicate that in malaria-endemic countries of Africa, children admitted with severe anaemia and severe malnutrition remain at a high risk of dying in the first few months post-hospitalisation. The World Health Organization (WHO) has developed guidelines for the post-discharge management and follow up of children admitted with severe malnutrition; similar guidelines are now needed for children admitted with severe anaemia in malaria-endemic areas of Africa.[44]

Although the risk of post-discharge mortality was highest among those admitted with severe anaemia and malnutrition, the review also showed that any hospitalised child, regardless of admission syndrome, was at increased risk of dying during the first six months post-discharge and the average mortality was approximately 3-fold higher during this period compared to apparently normal, un-hospitalised children in the same community. These findings are in agreement with a recent systematic review of all-cause post-discharge mortality among the general paediatric population, which indicated a significant global burden of post-discharge mortality, especially in low-income countries.[18]

Recently the term “post-hospital syndrome” was proposed, which refers to an acquired, transient condition of vulnerability in recently hospitalized, mostly elderly patients in the United States of America resulting in a period of generalized risk for myriad adverse health events not necessarily linked to the original illness. During this period the patient is not only recovering from the original acute illness but also suffers continued physiological and immunological impairment due to the initial acute illness and other stressors following hospitalisation.[45]

We were unable to analyse the reasons for the high post-discharge mortality or re-admission rates observed in children with severe anaemia because of insufficient data, but this is likely to reflect the complex multifactorial nature of the aetiology of anaemia in this setting[46] and the continued exposure to the same risk factors for severe anaemia in the community that resulted in the initial admission, and possible delays in seeking appropriate care due to local beliefs and perceptions about severe anaemia.[47] Although data on HIV status was not reported as a standalone syndrome on admission, HIV-positive children were at 4- fold increased risk of post-discharge mortality compared to those who are HIV negative. Recurrent malaria infections have also been reported as risk factors for recurrence of severe anaemia among these children.[19,20,41]

Malnourished children were not only at increased risk of post-discharge mortality compared to other children, but the co-existence of undernutrition was also reported as a significant contributor to post-discharge mortality among children recently admitted with other syndromes.[23,32,35,39] A greater proportion of undernourished children in developing countries have an underlying medical condition either directly causing or significantly contributing to malnutrition.[15] By contrast, to severe anaemia, malnutrition is recognised as a major risk factor for post-discharge mortality in resource-poor countries with guidelines for further follow-up and care at home and periodic monitoring to avoid relapse.[22,48,49]

Children with severe malaria per se (e.g. cerebral malaria) had lower post-discharge mortality than children with severe anaemia. We also found a significantly lower risk of post-discharge mortality among children with severe malarial anaemia compared to children with severe anaemia without malaria. It is possible that the attribution of multiple and more chronic causes of severe anaemia such as micro-nutrient deficiencies or chronic infections such as tuberculosis and HIV was greater among the children without malaria. They would require multiple and long-term interventions post-discharge. This may not be the case for children with malaria as the main cause of severe anaemia, in particular, if the initial event was successfully treated with blood transfusion and antimalarials. Alternatively, the in-patient population treated for 'severe' malaria may reflect a heterogeneous group of patients as in many countries large numbers of uncomplicated malaria patients are admitted as in-patients to health facilities.[50]

Although in-hospital severe malaria was not a major risk factor for post-discharge death, in highly endemic areas, malaria may become an important risk factor for readmissions or mortality post-discharge in all groups, as children experience new or recrudescent malaria infections after discharge[3,41] leading to continued dyserythropoietic and bone marrow

suppression that may last for several weeks.[51,52] In children initially admitted with severe anaemia this may negate the initial rise in Hb achieved by blood transfusion leading to slow haematological recovery resulting in rebound severe anaemia, poor immunological response to bacterial and other infections and even death.

Other significant factors reported across studies irrespective of the initial exposure syndrome include; hypoxia, bacteraemia/sepsis, jaundice, hepatomegaly, splenomegaly, prolonged hospitalisation, lower socio-economic status, reduced consciousness on admission (Blantyre coma scale <5), delay in seeking care, history of previous hospitals admissions, young age and discharge against medical advice.

These findings support the need for post-discharge management strategies for recently discharged children especially those with severe anaemia and severe acute malnutrition in malaria-endemic areas of Africa. Post-discharge Interventions that have been shown to be effective include; preventive zinc supplementation to reduce morbidity and mortality due to diarrhoea and pneumonia,[53] prophylaxis with co-trimoxazole to reduce morbidity and mortality in HIV-positive individuals. Evidence from Malawi shows that 3 months of Post-discharge Malaria Chemoprevention (PMC) prevented 31% of deaths and readmissions in recently discharged children with severe malarial anaemia.[43] A confirmatory PMC trial comparing 3 monthly full courses of PMC with dihydroartemisinin-piperaquine against placebo was recently concluded in Uganda and Kenya.[54]

The findings from this review will also be used to model the potential post-discharge mortality and morbidity among children recently hospitalised with severe anaemia to estimate the potential impact of PMC in different malaria-endemic areas in sub-Saharan Africa.

There are important limitations to this type of secondary analysis that should be considered. Some limitations are common to many meta-analyses. First, there was considerable variation in the duration and/or reporting of follow-up data; e.g. some studies followed children only for one month, whereas others followed them for much longer than 6 months but did not report results by 6 months and could thus not contribute to our primary analysis. Second, variations in the design of observational studies and RCTs and their reporting contributed to observed heterogeneity. Third, few studies reported both on in-hospital and post-discharge mortality, and few studies reported post-discharge mortality by admission syndrome, and those that did had relatively modest sample sizes and the number of events. Fourth, we were not able to analyse the causes of post-discharge deaths due to insufficient data. Fifth, included studies that recruited children with severe anaemia did not report on

the aetiology of anaemia on admission, therefore we could not determine cause-specific severe anaemia mortality burden in-hospital or link the aetiology to post-discharge mortality burden. The inconsistency in reporting the risk factors for post-discharge mortality and scarcity of data on the causes of post-discharge mortality is an important consideration for future research necessary to develop a comprehensive post-discharge management plan.

Conclusions

This review reveals the scarcity of data on post-discharge morbidity and mortality. Despite the small number of studies, it confirms that children <5 years of age recently hospitalised with severe anaemia or severe malnutrition who survive the initial in-hospital period are at excess risk of dying during the first six months post-discharge compared to children admitted for other causes such as malaria. There is a need to develop post-discharge management strategies for these high-risk groups.

Article Information

Contributors

TKK and FOtK conceived the idea. TKK wrote the protocol with input from ATM, AVE, SN and FOtK. TKK and AMT developed the search terms and applied to the electronic databases. TKK and ATM reviewed all abstracts and selected full-text articles selected and assigned bias scores; FOtK served as the tiebreaker. TKK and ATM independently abstracted all the data. TKK conducted the meta-analysis with inputs from FOtK and SN. SN provided statistical support. TKK and FOtK wrote the first draft of the manuscript. All authors reviewed, revised and approved the final version of the manuscript.

Declaration of interests

There are no conflicts of interest to declare.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to:

Titus K Kwambai, Amani T Mori, Sarah Nevitt, Annemieke van Eijk, Bjarne Robberstad, Kamija Phiri, Feiko O ter Kuile.

Post-discharge risks of morbidity and mortality in children admitted with severe anaemia and other syndromes in malaria-endemic settings in Africa: A systematic review and meta-analysis

Supplemental methods

Supplement 1: Search terms used in PubMed

((((((((child) OR childhood) OR infant) OR paediatric)) AND (((((((((anemia) OR "severe anemia") OR "severe malaria") OR "severe malaria anemia") OR "severe malarial anemia") OR "severe malarial anaemia") OR "severe pneumonia") OR "severe malnutrition") OR diarrhea) OR sepsis)) AND ((((((death) OR admission) OR readmission) OR mortality) OR morbidity) OR survival) OR post-discharge syndrome)) AND (((((post-discharge) OR post-discharge) OR post hospitalization) OR longitudinal) OR follow-up) OR discharge follow-up)) AND ((cape verde) OR Gambia) OR Mauritania) OR guinea bissau) OR liberia) OR togo) OR sierra leone) OR benin) OR senegal) OR guinea) OR cote d'ivoire) OR mali) OR ghana) OR burkina fasso) OR niger) OR nigeria) OR zimbabwe) OR zambia) OR swaziland) OR south africa) OR namibia) OR mozambique) OR malawi) OR mauritius) OR madagascar) OR botswana) OR angola) OR egypt) OR uganda) OR tanzania) OR sudan) OR somalia) OR rwanda) OR mayotte) OR kenya) OR ethiopia) OR eritrea) OR djibouti) OR comoros) OR burundi) OR "sao tome") OR ("sao tome and principe")) OR "equatorial guinea") OR gabon) OR "central african republic") OR congo) OR chad) OR cameroon) OR "democratic republic of congo")) OR ((africa) OR sub-saharan africa))) NOT ((((((("aplastic anemia") OR "severe aplastic anemia") OR "acquired aplastic anemia") OR "Fanconi anemia") OR "megaloblastic anemia") OR "maternal anemia") OR "pregnant women") OR "sickle cell anemia").

Supplement 2: Outcome definitions

Primary outcomes

All-cause death or all-cause readmission in the first six months post-discharge

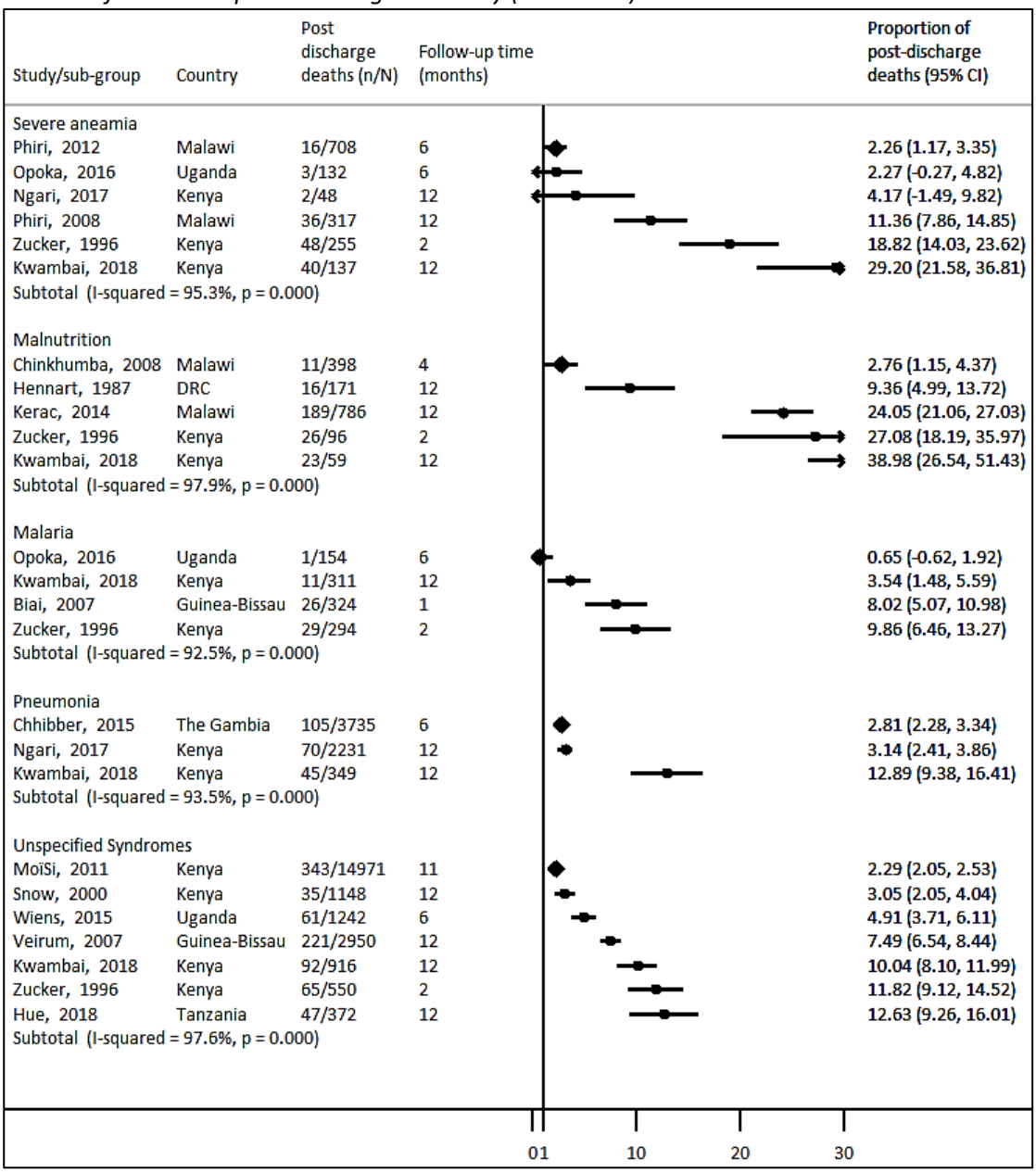
Secondary outcomes

- Deaths due to severe anaemia in the first six months post-discharge
- Deaths due to syndromes other than severe anaemia in the first six months post-discharge
- In-hospital deaths due to severe anaemia
- In-hospital deaths due to syndromes other than severe anaemia

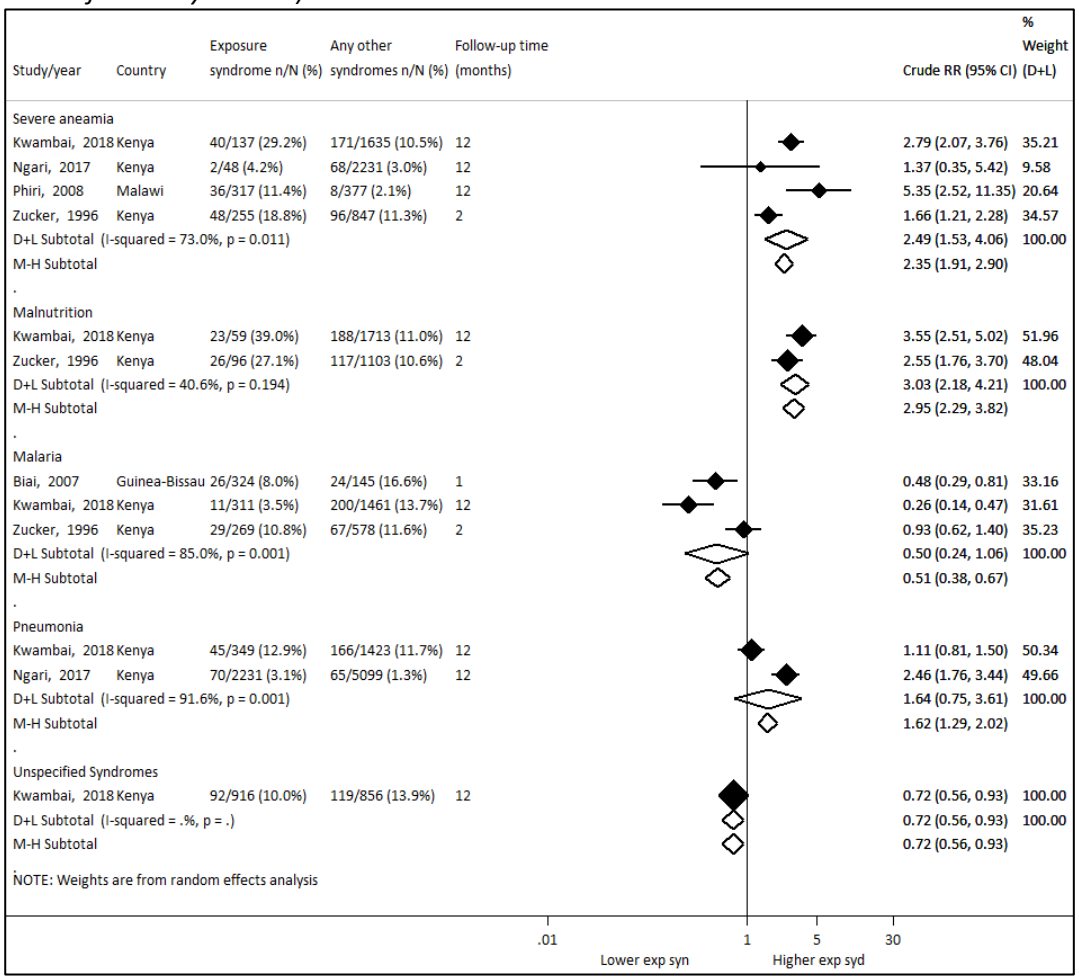
- Readmission due to all-cause severe anaemia (defined as Hb <5 g/dL or PCV <15% or requirement for blood transfusion based on other clinical indication irrespective of the Hb level).
- Readmission due to syndromes other than severe anaemia

Supplemental Figures

eFigure 1: Mortality by twelve months post-discharge
1a. Risk of 12-month post-discharge mortality (all studies).



1b. Relative Risk of 12-month post-discharge mortality (excludes studies that only reported results for one syndrome).

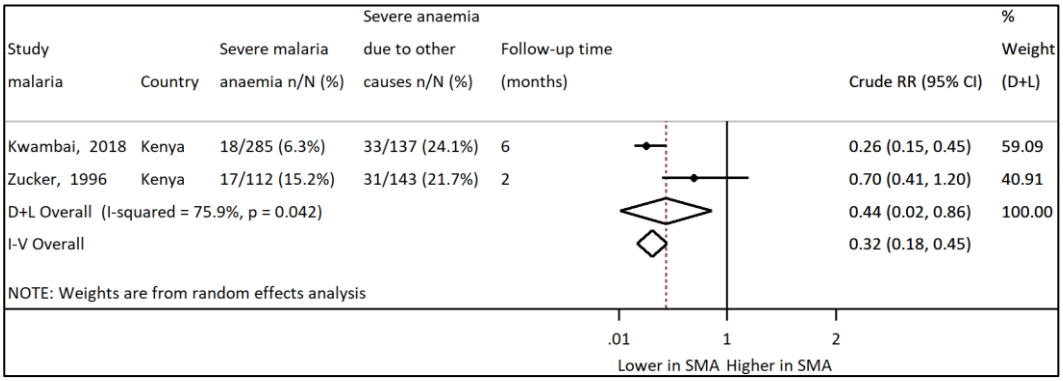


RR, relative risk; CI, confidence interval; D+L, DerSimonian and Laird; M-H, Mantel-Haenszel.

Top panel a: Kwambai 2018, Zucker 1996 and Opoka, 2016 are included in more than one sub-group each representing a mutually exclusive group. The lower CI for Opoka, 2016 and Ngari, 2017 are negative due to the small number of post-discharge deaths reported, the lower CIs should be treated as zero. Due to considerable heterogeneity (as shown by the I^2statistic) between and within admission syndrome groups the summary statistics are not shown. The pooled I^2 is 96.6%.

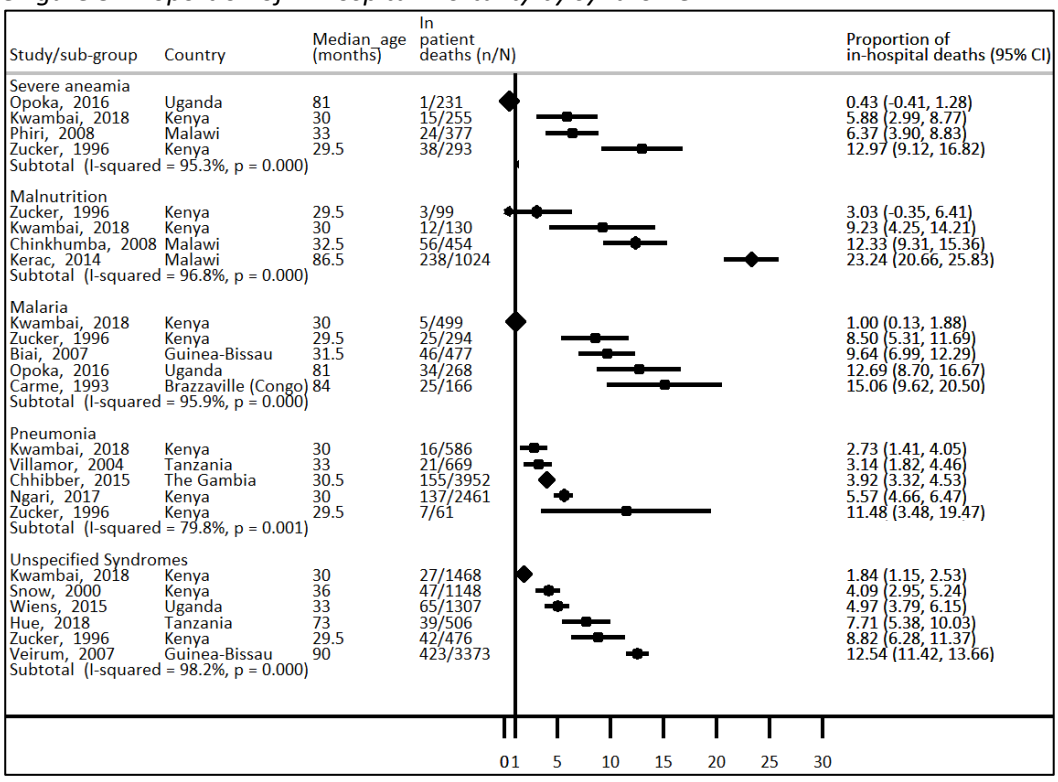
Bottom panel b: Includes only studies that reported enough detail to allow direct comparisons of the post-discharge mortality by syndrome among children from the same cohort study.

eFigure 2: Post-discharge mortality between severe malaria anaemia vs severe anaemia due to other causes.



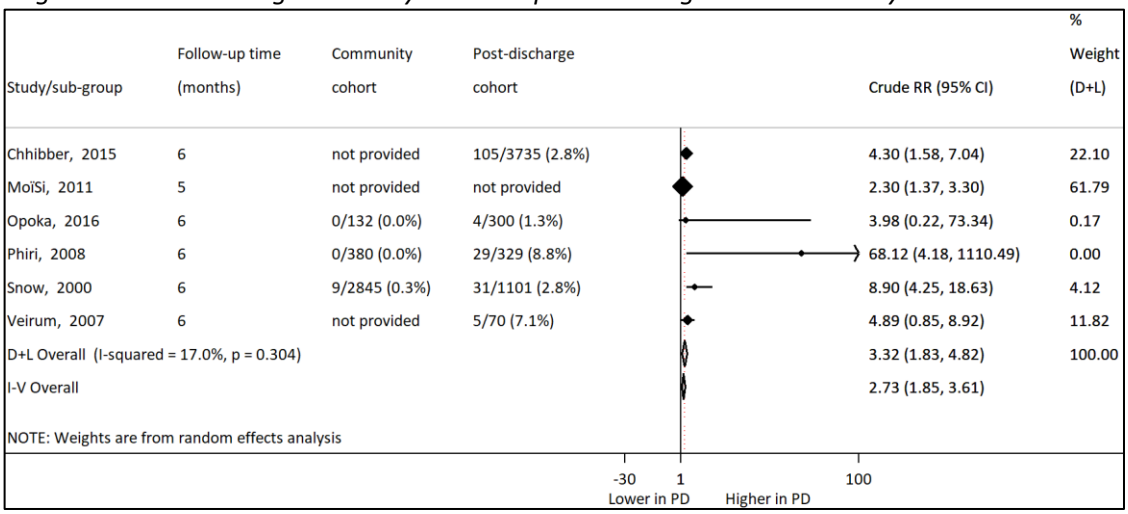
CI, confidence interval; SMA, severe malarial anaemia; D+L, DerSimonian and Laird; MHOR, Mantel-Haenszel.

eFigure 3: Proportion of in-hospital mortality by syndrome.



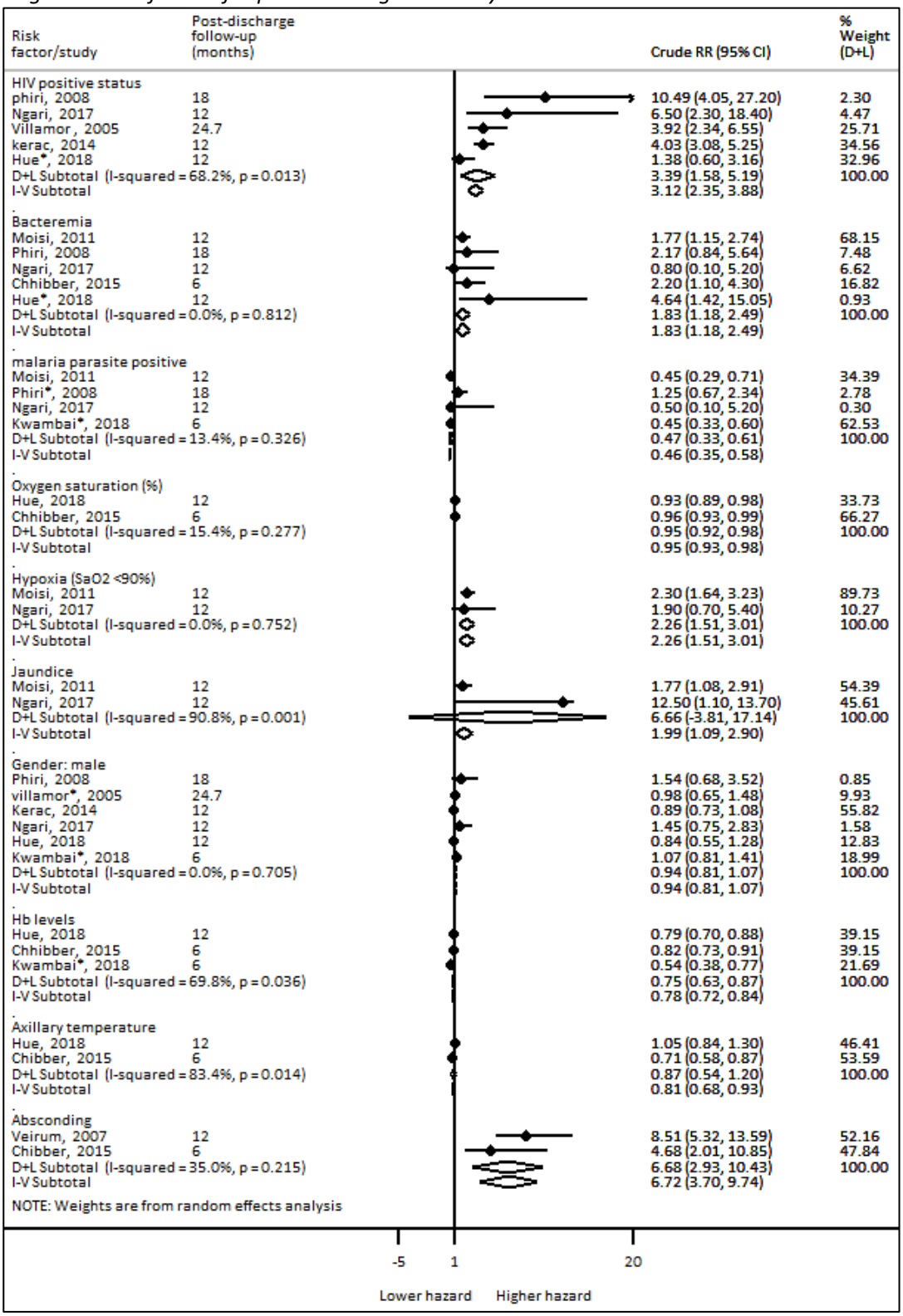
Kwambai 2018, Zucker 1996 and Opoka, 2016 are included in more than one sub-group each representing a mutually exclusive group. The lower CI for Opoka, 2016 and Zucker, 1996 are negative due to the small number of post-discharge deaths reported, the lower CIs should be treated as zero. Due to considerable heterogeneity (as shown by the I^2statistic) between and within admission syndrome groups the summary statistics are not shown. The pooled I^2 is 96.9%.

eFigure 4: Post-discharge mortality between post-discharge and community cohorts.



RR, relative risk; CI, confidence interval; PD, post-discharge.

eFigure 5: Risk factors for post-discharge mortality



CI, confidence interval; HR, hazard ratio; Hb, haemoglobin
*Unadjusted HR

Supplemental tables

eTable 1: Cochrane collaboration tool for quality assessment of randomised controlled trials

	Random Sequence Generation	Allocation Concealment	Blinding of Participants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Reporting	Other Bias
Biai et al, 2007	+	+	+	+	+	+	+
Phiri et al, 2012	+	+	+	+	+	+	+

+	Low Risk of Bias	?	Unclear Risk of Bias	-	High Risk of Bias

Risk of bias assessment for included studies with authors' judgements for each included trial. Adapted from the Cochrane Library.

eTable 2: Newcastle Ottawa scale for quality assessment of cohort studies with comparison groups

	(summary of reviewers scores [*=1, no*=0])								
	Study								
Criterion	Chinkhumba 2008	Kwambai 2018	Ngari 2017	Opkoka 2016	Veirum 2007	Zucker 1996	Snow 2000	Moisi 2011	Phiri 2008
Selection									
Representativeness of exposed cohort	1	1	1	1	1	1	1	1	1
selection of the non-exposed cohort	1	1	1	1	1	1	1	1	1
Ascertainment of exposure	1	1	1	1	1	1	1	1	1
Demonstration that the outcome of interest was not present at the start of the study	1	1	1	1	1	1	1	1	1
Comparability									
Comparability of cohorts based on the design or analysis	2	2	2	2	1	1	1	2	2
Outcome									
Assessment of outcome	1	1	1	1	1	1	1	1	1
Was follow up long enough for outcomes to occur?	1	1	1	1	1	1	1	1	1
Adequacy of follow up of cohorts	0	1	0	1	0	0	0	0	1
Total starts awarded out of 9	8	9	8	9	7	7	7	8	9
Quality Assessment	Good	Good	Good	Good	Good	Good	Good	Good	Good

The Newcastle Ottawa scale is based on a star system where one star is awarded for each item under selection and outcome categories and a maximum of two stars for comparability. A maximum of nine points is assigned for the least risk of bias in three domains: 1) selection of study groups (four points); 2) comparability of groups (two points); and 3) ascertainment of outcomes (three points) for cohort studies. The quality of studies was rated based on the Newcastle Ottawa scale of 0 to 9 as; poor quality (0 -3), fair quality (4 -6) and good quality (7 -9).

eTable 3: Modified Newcastle Ottawa scale for quality assessment of cohort studies without comparison

CRITERION	**summary of reviewers scores [*=1, no*=0]** **Study**						
	Carme 1993	Chhibber 2015	Hennart 1987	Kerac 2014	Villamor 2014	Wiens 2015	Hue 2018
Selection							
Representativeness of exposed cohort	1	1	1	1	1	1	1
selection of the non-exposed cohort	NA	NA	NA	NA	NA	NA	NA
Ascertainment of exposure	1	1	1	1	1	1	1
Demonstration that outcome of interest was not present at the start of the study	0	1	1	1	1	0	1
Comparability							
Comparability of cohorts on the basis of the design or analysis	NA	NA	NA	NA	NA	NA	NA
Outcome							
Assessment of outcome	0	1	1	1	1	1	1
Was follow up long enough for outcomes to occur?	1	1	1	1	1	1	1
Adequacy of follow up of cohorts	0	1	0	1	1	1	1
Total starts awarded out of 6	3	6	5	6	6	5	6
Quality Assessment	Poor	Good	Good	Good	Good	Good	Good

This tool was used for cohort studies without a comparison group. A single group of participants with an exposure of interest were followed up over to determine the outcome. In the "selection" criteria of the Newcastle Ottawa scale, the "selection of the non-exposed cohort" and the comparability criteria were omitted. The total score is six stars with quality assessment scored as; 1-2 stars (poor quality), 3-4 (fair quality) and 5-6 (good quality).

eTable 4: Risk factors for post-discharge mortality

Author	Risk factor	Effect Measure (EM)	EM (95% CI)	Adjusted
Moisi34	Age 1–5 months (ref: <1 month)	HR	1.34 (0.93-1.92)	Yes
	Age 6–11 months (ref: <1 month)	HR	0.82 (0.57-1.18)	Yes
	Age 2–5 years (ref: <1 month)	**HR**	**0.57 (0.36-0.90)**	**Yes**
	Weigh for age z-score < −3	**HR**	**3.42 (2.50-4.68)**	**Yes**
	Weigh for age z-score < −4	**HR**	**6.53 (4.85-8.80)**	**Yes**
	Parasitaemia (ref: no parasitaemia)	**HR**	**0.45 (0.29-0.71)**	**Yes**
	Hypoxia (ref: no hypoxia)	**HR**	**2.3 (1.64-3.23)**	**Yes**
	Bacteraemia (ref: no bacteraemia)	**HR**	**1.77 (1.15-2.74)**	**Yes**
	Jaundice (ref: no jaundice)	**HR**	**1.77 (1.08-2.91)**	**Yes**
	Hepatomegaly (ref: no hepatomegaly)	**HR**	**2.34 (1.60-3.42)**	**Yes**
	Hospitalization > 13 d (ref: <13 days)	**HR**	**1.83 (1.33-2.52)**	**Yes**
	1 prior discharge (within a 1 year of index discharge) (ref: no prior discharge)	**HR**	**2.83 (2.04-3.92)**	**Yes**
	2 prior discharges (within a 1 year of index discharge) (ref: no prior discharge)	**HR**	**7.06 (4.09-12.21)**	**Yes**
	≥ 3 prior discharges (within a 1 year of index discharge) (ref: no prior discharge)	**HR**	**23.55 (10.70-51.84)**	**Yes**
	Mild pneumonia (ref: no pneumonia)	**HR**	**2.3 (1.00-5.28)**	**Yes**
	Severe pneumonia (ref: no pneumonia)	**HR**	**1.37 (1.05-1.79)**	**Yes**
	Very severe pneumonia (ref: no pneumonia)	**HR**	**4.09 (2.25-7.46)**	**Yes**
	Severe malnutrition (ref: no malnutrition)	**HR**	**4.37 (2.73-7.01)**	**Yes**
	Meningitis (ref: no meningitis)	**HR**	**2.29 (1.57-3.32)**	**Yes**
	Sick young infant	**HR**	**2.67 (1.98-3.58)**	**Yes**
Wiens39	Male sex (ref: female)	OR	0.90 (0.54-1.51)	No
	Age (months) (per unit increase)	OR	0.97 (0.97-0.97)	No
	MUAC (per mm increase)	**OR**	**0.97 (0.96-0.98)**	**No**
	Weight for age z-score (per unit increase)	**OR**	**0.66 (0.57-0.76)**	**No**
	Weight for length/height z-score (per unit increase)	**OR**	**0.81 (0.72-0.91)**	**No**

Author	Risk factor	Effect Measure (EM)	EM (95% CI)	Adjusted
	Length/height for age z-score (per unit increase)	**OR**	**0.79 (0.7 -0.89)**	**No**
	Heart rate for age z-score	**OR**	**0.86 (0.74-0.99)**	**No**
	Heart rate (raw)	OR	1.00 (0.99-1.01)	No
	Respiratory rate for age z-score	OR	0.99 (0.92-1.06)	No
	Respiratory rate (raw)	**OR**	**1.01 (1.00-1.03)**	**No**
	Systolic blood pressure z-score	OR	0.94 (0.79-1.12)	No
	Systolic blood pressure (raw)	OR	0.98 (0.96-1.00)	No
	Diastolic blood pressure (raw)	OR	0.99 (0.97-1.01)	No
	Temperature (transformed)	OR	1.02 (0.90-1.16)	No
	Temperature (raw)	**OR**	**0.76 (0.62-0.93)**	**No**
	SpO2 (raw) (per 1% increase)	**OR**	**0.94 (0.92-0.96)**	**No**
	SpO2 (transformed) (per 1% increase)	**OR**	**1.04 (1.02-1.05)**	**No**
	HIV positive (ref: negative)	**OR**	**5.21 (2.55-10.65)**	**No**
	Hb (g/dL)	OR	0.95 (0.87-1.03)	No
	Blantyre Coma Scale <5 (ref: 5)	**OR**	**2.40 (1.27-4.57)**	**No**
	Positive blood smear (vs negative)	**OR**	**0.33 (0.16-0.68)**	**No**
	Illness >7 days prior to admission	**OR**	**0.50 (0.30-0.83)**	**No**
	Time since last hospitalisation (Ordered as <7 days, 7 to 30 days, 30 days to 1 year, >1 year and never (analysed as continuous and coded and 1–5, respectively)	**OR**	**0.75 (0.62-0.90)**	**No**
	Sibling deaths	OR	1.54 (0.89-2.65)	No
	Number of children in family	OR	1.02 (0.92-1.13)	No
	Boil all drinking water	OR	0.82 (0.47-1.42	No
	Maternal age (years)	OR	1.00 (0.97-1.04)	No
	Mother HIV positive (ref: negative)	OR	1.79 (0.87-3.67)	No
	Mother HIV status unknown (ref: negative))	OR	1.27 (0.64-2.52)	No
	Maternal education (Primary) (ref: <primary 3)	OR	1.18 (0.62-2.23)	No

Author	Risk factor	Effect Measure (EM)	EM (95% CI)	Adjusted
	Maternal education (Some) (ref: <primary 3)	OR	0.72 (0.31-1.70)	No
	Maternal education (Postsecondary) (ref: <primary 3)	OR	1.18 (0.41-3.36)	No
	Bed net use (Sometimes) (ref: never)	OR	1.00 (0.48-2.09)	No
	Bed net use (always) (ref: never)	OR	0.85 (0.46-1.58)	No
	Distance from hospital (30–60 minutes) (ref: <30 min)	OR	0.71 (0.31-1.64)	No
	Distance from hospital (>60 minutes) (ref: <30 min)	OR	1.30 (0.70-2.41)	No
Phiri20	Unit increase in age (months)	**HR**	**0.92 (0.87-0.97)**	**Yes**
	Rural residency (ref: urban)	HR	1.63 (0.63-4.20)	Yes
	Sex (Male)	HR	1.54 (0.68-3.52)	Yes
	Maternal education (Some) (ref: none)	HR	1.63 (0.72-3.70)	No
	Parents unemployed (ref: employed)	**HR**	**4.15 (1.61-10.74)**	**Yes**
	Weight-for-height <-2 Z-score (≥-2 Z-score WHZ)	HR	0.74 (0.31-1.80)	No
	Height-for-age <-2 Z-score (≥-2 Z-score HAZ)	HR	0.61 (0.30-1.22)	No
	Splenomegaly (ref: no splenomegaly)	**HR**	**0.36 (0.16-0.80)**	**Yes**
	Iron deficiency ≥5.6 sTfR/Log ferritin (ref: <5.6 sTfR/Log ferritin)	HR	0.91 (0.41-2.03)	No
	Any malaria parasite/µL blood (ref: no parasitaemia)	HR	1.25 (0.67-2.34)	No
	HIV Positive (ref: HIV negative)	**HR**	**10.49 (4.05-27.20)**	**Yes**
	Bacteraemia (ref: no bacteraemia)	HR	2.17 (0.84-5.64)	Yes
Ngari35	Age 12–23 months (ref: ≥24 months)	HR	1.02 (0.1-9.6)	Yes
	Age 6–11 months (ref: ≥24 months)	HR	5.8 (0.8-40.5)	Yes
	Age <6 months (ref: ≥24 months)	HR	4.8 (0.7-34.1)	Yes
	Sex (male)	HR	1.45 (0.75-2.83)	Yes
	Reported preterm/low birthweight (LBW) (ref: no preterm/LBW)	HR	0.7 (0.2-2.8)	Yes
	Residence distance (from study site per KM)	HR	1.0 (0.9-1.1)	Yes
	Duration of hospitalisation per day	**HR**	**1.1 (1.0-1.2)**	**Yes**
	Hypoxia (SaPO2 <90%) (ref: SaPO2 >90%)	HR	1.9 (0.7-5.4)	Yes

Author	Risk factor	Effect Measure (EM)	EM (95% CI)	Adjusted
	Capillary refill >2 seconds (ref: <2seconds)	HR	2.4 (0.5-12.1)	Yes
	Impaired consciousness (ref: normal consciousness)	HR	1.1 (0.2-7.8)	Yes
	Wheezing (ref: no wheezing)	HR	0.5 (0.1-2.4)	Yes
	Cough for >14 days	HR	0.2 (0.1-5.5)	Yes
	Jaundice (ref: no jaundice)	**HR**	**12.5 (1.1-13.7)**	**Yes**
	Severe anaemia (Hb <5g/dL) (ref: Hb≥5)	HR	0.8 (0.1-7.5)	Yes
	Axillary temperature <36°C (ref: axillary temperature 36 to 39oc)	HR	0.3 (0.1-2.8)	Yes
	Axillary temperature >39°C (ref: axillary temperature 36 to 39oc)	HR	1.1 (0.4-3.0)	Yes
	HIV antibody test positive (ref: HIV negative)	**HR**	**6.5 (2.3-18.4)**	**Yes**
	HIV test not performed (ref: HIV negative)	HR	0.4 (0.1-3.6)	Yes
	Respiratory Syncytial Virus test positive (ref: RSV test negative)	HR	0.3 (0.1-1.2)	Yes
	Respiratory Syncytial Virus test not performed (ref: RSV test negative)	**HR**	**2.7 (1.2-6.3)**	**Yes**
	Malaria slide positive (ref: negative)	HR	0.5 (0.1-5.2)	Yes
	Bacteraemia (ref: no bacteraemia)	HR	0.8 (0.1-5.2)	Yes
	MUAC per cm increase	**HR**	**0.6 (0.5-0.8)**	**Yes**
	Year of admission 2008 (ref: Year of admission 2007)	HR	0.9 (0.3-3.1)	Yes
	Year of admission 2009 (ref: Year of admission 2007)	HR	0.5 (0.1-2.1)	Yes
	Year of admission 2010 (ref: Year of admission 2007)	HR	0.7 (0.2-2.5)	Yes
	Year of admission 2011 (ref: Year of admission 2007)	HR	1.7 (0.5-5.3)	Yes
	Year of admission 2012 (ref: Year of admission 2007)	HR	1.8 (0.2-15.7)	Yes
Villamor38	HIV Positive (ref: HIV negative)	**HR**	**3.92 (2.34-6.55)**	**Yes**
	Sex Male	HR	0.98 (0.65-1.48)	No
	Age 6–11 months (ref: ≥24 months)	**HR**	**3.70 (1.72-7.95)**	**Yes**
	Age 12–23 months (ref: ≥24 months)	**HR**	**3.14 (1.44-6.88)**	**Yes**
	Height-for-age <-2 Z-score (ref: HAZ>-2 Z-score)	**HR**	**2.12 (1.31-3.42)**	**Yes**

Author	Risk factor	Effect Measure (EM)	EM (95% CI)	Adjusted
	Low MUAC at baseline (MUAC <25th percentile of the population age-specific distribution) (per cm increase)	**HR**	**1.88 (1.16-3.03)**	**Yes**
	Hb ≤7.00 g/dL (ref: Hb >10.00g/dL)	**HR**	**2.55 (1.13-5.77)**	**Yes**
	Hb 7.01–8.50 g/dL (ref: Hb >10.00g/dL)	**HR**	**2.81 (1.24-6.37)**	**Yes**
	Hb 8.51–10.00 g/dL (ref: Hb >10.00g/dL)	HR	1.76 (0.75-4.10)	Yes
	Severe pneumonia on admission (ref: no pneumonia on admission)	**HR**	**2.47 (1.59-3.85)**	**Yes**
	Maternal education (Elementary) (ref: None/illiterate)	HR	0.84 (0.48-1.49)	No
	Maternal education (Secondary or higher) (ref: None/illiterate)	HR	0.27 (0.06-1.17)	No
	Tap in compound (ref: tap in house)	HR	1.40 (0.60-3.29)	Yes
	Tap outside compound (ref: tap in the house)	**HR**	**2.27 (1.02-5.03)**	**Yes**
	Public well (ref: tap in the house)	**HR**	**2.92 (1.03-8.30)**	**Yes**
	Mother works outside home-yes (ref: no)	HR	0.61 (0.36-1.03)	No
	Mother lives with a partner (ref: mother lives without a partner)	**HR**	**1.60 (1.00-2.57)**	**No**
	No household amenity (ref: 1 household amenity) (from a list of five items: car, refrigerator, radio, bicycle, and television)	HR	1.58 (0.92-2.69)	No
	2≤ household amenities (ref: 1 household amenity)	HR	0.95 (0.56-1.60)	No
Kerac23	Sex (Male)	HR	0.89 (0.73-1.08)	Yes
	Age >=60 months (ref: age 48 to 60 months)	HR	1.22 (0.63-2.36)	Yes
	Age 36 to 48 months (ref: age 48 to 60 months)	HR	1.66 (0.84-3.29)	Yes
	Age 24 to 36 months (ref: age 48 to 60 months)	HR	1.38 (0.76-2.49)	Yes
	Age 12 to 24 months (ref: age 48 to 60 months)	HR	1.57 (0.89-2.78)	Yes
	Age <12 months (ref: age 48 to 60 months)	**HR**	**2.49 (1.38-4.51)**	**Yes**
	Oedema (ref: no oedema)	**HR**	**0.58 (0.47-0.72)**	**Yes**
	MUAC per cm increase	**HR**	**0.80 (0.74-0.86)**	**Yes**
	weight-for-height (per 1-unit z-score increase)	**HR**	**0.75 (0.68-0.83)**	**Yes**
	Weight for age (per 1-unit z-score increase)	**HR**	**0.73 (0.66-0.81)**	**Yes**
	height for age z-score (per 1-unit z-score increase)	**HR**	**0.92 (0.86-0.99)**	**Yes**

Author	Risk factor	Effect Measure (EM)	EM (95% CI)	Adjusted
	HIV Positive (ref: HIV negative)	**HR**	**4.03 (3.08-5.25)**	**Yes**
	HIV status unknown (ref: HIV negative)	**HR**	**16.9 (12.1-23.7)**	**Yes**
Chhibber31	Sepsis with clinically severe malnutrition (ref: pneumonia without clinically severe malnutrition)	**HR**	**18.4 (11.3-30.0)**	**Yes**
	Meningitis with clinically severe malnutrition (ref: pneumonia without clinically severe malnutrition)	**HR**	**13.7 (4.2-44.7)**	**Yes**
	Pneumonia with clinically severe malnutrition (ref: pneumonia without clinically severe malnutrition)	**HR**	**8.1 (4.4-14.8)**	**Yes**
	Meningitis without clinically severe malnutrition (ref: pneumonia without clinically severe malnutrition)	**HR**	**2.6 (1.2-5.5)**	**Yes**
	Sepsis without clinically severe malnutrition (ref: pneumonia without clinically severe malnutrition)	**HR**	**2.2 (1.1-4.3)**	**Yes**
	Age in months (mean [SD])	HR	1.00 (0.98-1.03)	Yes
	Neck stiffness (ref: no neck stiffness)	**HR**	**10.4 (3.1-34.8)**	**Yes**
	Non-medical discharge (i.e. discharge against medical advice) (ref: medical discharge)	**HR**	**4.68 (2.01-10.85)**	**Yes**
	Axillary temperature (oC) (mean [SD])	**HR**	**0.71 (0.58-0.87)**	**Yes**
	Oxygen saturation (% increase)	**HR**	**0.96 (0.93-0.99)**	**Yes**
	Hb in g/dL (mean [SD])	**HR**	**0.82 (0.73-0.91)**	**Yes**
	MUAC 11.5–13.0 cm (ref: MUAC>13cm)	**HR**	**7.19 (3.04-17.01)**	**Yes**
	MUAC 10.5–11.4 cm (ref: MUAC>13cm)	**HR**	**24.2 (9.4-61.9)**	**Yes**
	MUAC <10.5 cm (ref: MUAC>13cm)	**HR**	**43.7 (17.7-108)**	**Yes**
Veirum37	Discharge age 5 years+ (ref: 1-12 months)	**RR**	**0.15 (0.05-0.30)**	**Yes**
	Discharge age 4 years (ref: 1-12 months)	**RR**	**0.23 (0.09-0.48)**	**Yes**
	Discharge age 3 years (ref: 1-12 months)	**RR**	**0.14 (0.04-0.39)**	**Yes**
	Discharge age 2 years (ref: 1-12 months)	**RR**	**0.52 (0.25-0.76)**	**Yes**
	Discharge age 1 year (ref: 1-12 months)	RR	0.82 (0.67-1.41)	Yes
	Neonatal (ref: 1-12 months)	RR	0.69 (0.21-1.77)	Yes
	Mothers' education (ref: no education)	RR	0.74 (0.56-1.14)	Yes

Author	Risk factor	Effect Measure (EM)	EM (95% CI)	Adjusted
	Non-medical discharge (against medical advice) (ref: medical discharge)	**RR**	**8.51 (5.32-13.59)**	**Yes**
	Other (ref: malaria)	**RR**	**1.65 (1.02-2.92)**	**Yes**
	Anaemia (ref: malaria)	RR	1.97 (0.97-4.00)	Yes
	Diarrhoea (ref: malaria)	RR	1.82 (0.83-2.35)	Yes
	Bronchopneumonia (ref: malaria)	RR	0.98 (0.66-1.74)	Yes
	Measles (ref: malaria)	RR	0.77 (0.43-2.22)	Yes
Hue et al41	Age 5 – 12 years (ref: <5 years)	**HR**	**1.75 (1.15-2.68)**	**No**
	Age 5 – 12 years (ref: <5 years)	**HR**	**1.01 (1.00-1.01)**	**Yes**
	Pit latrine at home (ref: none)	**HR**	**1.58 (1.00-2.50)**	**No**
	Sex (ref: female)	HR	0.84 (0.55-1.28)	No
	Lake or pond as a water source (ref: no)	HR	1.10 (0.71-1.69)	No
	HIV Status (HIV negative)	HR	1.38 (0.60-3.16)	No
	Decreased urine output (ref: no)	**HR**	**4.95 (2.83-8.66)**	**No**
	Diarrhea (ref: no diarrhoea)	HR	0.114 (0.39-1.11)	No
	Fever (ref: none)	HR	0.86 (0.54-1.36)	No
	Vomiting (ref: none)	HR	1.02 (0.63-1.63)	No
	Oxygen saturation: per % increase	**HR**	**0.93 (0.91-0.95)**	**No**
	Oxygen saturation: per % increase	**HR**	**0.93 (0.89-0.98)**	**Yes**
	Glasgow coma scale 13-14 (ref: <13)	**HR**	**0.66 (0.60-0.73)**	**No**
	Bilateral lower extremity edema (ref: none)	**HR**	**2.31 (1.40-3.81)**	**No**
	Respiratory Rate-bpm (per unit increase) 2-5 years	**HR**	**1.04 (1.02-1.06)**	**No**
	Respiratory Rate-bpm (per unit increase) 6-12 years	**HR**	**1.02 (1.00-1.04)**	**No**
	Diastolic blood pressure, mean mm Hg (per unit increase) 2-5 years	HR	0.99 (0.96-1.02)	No
	Diastolic blood pressure, mean mm Hg (per unit increase) 6-12 years	**HR**	**0.94 (0.91-0.98)**	**No**
	Heart rate, beats per minute, mean (SD) (per unit increase) 2-5 years	HR	1.00 (0.99-1.02)	No
	Heart rate, beats per minute, mean (SD) (per unit increase) 6-12 years	HR	1.01 (0.99-1.02)	No

Author	Risk factor	Effect Measure (EM)	EM (95% CI)	Adjusted
	Systolic blood pressure, mean mm Hg (per unit increase) 2-5 years	HR	1.00 (0.98-1.02)	No
	Systolic blood pressure, mean mm Hg (per unit increase) 6-12 years	HR	0.97 (0.95-1.00)	No
	Severe Malnutrition (ref: normal)	HR	1.49 (0.83-2.70)	No
	Moderate Malnutrition (ref: normal)	HR	1.07 (0.57-1.98)	No
	Mild Malnutrition (ref: normal)	HR	0.99 (0.55-1.77)	No
	Temperature, Celsius, mean (SD)	HR	1.05 (0.84-1.30)	No
	Hb level, g/dL, mean (SD) (per unit increase)	**HR**	**0.82 (0.75-0.88)**	**No**
	Hb level, g/dL, mean (SD) (per unit increase)	**HR**	**0.79 (0.70-0.88)**	**Yes**
	Proteinuria by urinalysis (ref: none)	**HR**	**2.38 (1.51-3.74)**	**No**
	Proteinuria by urinalysis (ref: none)	**HR**	**2.13 (1.12-4.05)**	**Yes**
	Hematuria by urinalysis (ref: none)	**HR**	**2.81 (1.35-5.81)**	**No**
	Glomerula filtration rate < 60 ml/min/1.73m2 (ref: no)	**HR**	**1.91 (1.21-3.02)**	**No**
	Random blood glucose, mg/dL, mean (SD) (per unit increase)	HR	0.98 (0.90-1.05)	No
	Cancer (ref: respiratory infections & malaria)	**HR**	**11.79 (4.95-28.03)**	**No**
	Heart disease (ref: respiratory infections & malaria)	**HR**	**7.11 (2.89-17.51)**	**No**
	Sickle cell disease (ref: respiratory infections & malaria	**HR**	**3.32 (1.44-7.68)**	**No**
	Neurologic diseases (ref: respiratory infections & malaria)	**HR**	**3.51 (1.35-9.11)**	**No**
	Septic shock (ref: respiratory infections & malaria)	**HR**	**4.64 (1.42-15.08)**	**No**
	Severe malnutrition (ref: respiratory infections & malaria)	**HR**	**3.19 (1.18-8.57)**	**No**
	Anemia (ref: respiratory infections & malaria)	HR	2.03 (0.75-5.46)	No
	Diarrheal diseases (ref: respiratory infections & malaria)	HR	1.94 (0.75-5.04)	No
	Diarrheal diseases (ref: respiratory infections & malaria)	HR	0.49 (0.20-1.17)	Yes
	Other (ref: respiratory infections & malaria)	HR	1.96 (0.73-5.27)	No
	Decreased urine output (ref: no)	**HR**	**4.95 (2.83-8.66)**	**No**
	Diarrhea (ref: no diarrhoea)	HR	0.114 (0.39-1.11)	No
	Fever (ref: none)	HR	0.86 (0.54-1.36)	No

Author	Risk factor	Effect Measure (EM)	EM (95% CI)	Adjusted
	Vomiting (ref: none)	HR	1.02 (0.63-1.63)	No
	Oxygen saturation: per % increase	**HR**	**0.93 (0.91-0.95)**	**No**

eTable 5: PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	90
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	91
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	92
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	93
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	92
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving a rationale.	93
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	93
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplement 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in a systematic review, and, if applicable, included in the meta-analysis).	93
Data collection process	10	Describe the method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	93 and 94
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	93 and 94

Risk of bias in individual studies	12	Describe methods used for assessing the risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	94
Summary measures	13	State the principal summary measures (e.g., risk ratio, the difference in means).	94
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	94
Section/topic	**#**	**Checklist item**	**Reported on page #**
Risk of bias across studies	15	Specify any assessment of the risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	94
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	94
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	94 and 95 and Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	94 and 95 and Figure 1
Risk of bias within studies	19	Present data on the risk of bias of each study and, if available, any outcome level assessment (see item 12).	95 and eTable 1 to eTable 3
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	99-101, 103, 104, 105, 116-120 eFigure 2 to 4 and supplemental content
Risk of bias across studies	22	Present results of any assessment of the risk of bias across studies (see Item 15).	eTable 1 to eTable 3
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	94
DISCUSSION			

Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policymakers).	106 to 107
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	108
Conclusions	26	Provide a general interpretation of the results in the context of other evidence and implications for future research.	109
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., the supply of data); the role of funders for the systematic review.	109 to 110

Chapter 5: Post-Discharge Malaria Chemoprevention Safety and Efficacy Study Design

Malaria Chemoprevention with Monthly Dihydroartemisinin-Piperaquine for The Post-Discharge Management of Severe Anaemia in Children Aged Less Than 5 Years in Uganda and Kenya: Study Protocol for A Multi-Centre, Two-Arm, Randomised, Placebo-Controlled, Superiority Trial.

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Abstract

Background

Children hospitalised with severe anaemia in malaria-endemic areas in Africa are at high risk of readmission or death within 6 months post-discharge. Currently, no strategy specifically addresses this period. In Malawi, 3 months of post-discharge malaria chemoprevention (PMC) with monthly treatment courses of artemether-lumefantrine given at discharge and at 1 and 2 months prevented 30% of all-cause readmissions by 6 months post-discharge. Another efficacy trial is needed before a policy of malaria chemoprevention can be considered for the post-discharge management of severe anaemia in children under 5 years of age living in malaria-endemic areas.

Objective

We aim to determine if 3 months of post-discharge malaria chemoprevention with monthly 3-day treatment courses of dihydroartemisinin-piperaquine is safe and superior to a single 3-day treatment course with artemether-lumefantrine provided as part of standard in-hospital care in reducing all-cause re-admissions and deaths (composite primary endpoint) by 6 months in the post-discharge management of children less than 5 years of age admitted with severe anaemia of any or undetermined cause.

Methods

This is a multi-centre, 2-arm, placebo-controlled, individually randomized trial in children under 5 years of age recently discharged following management for severe anaemia. Children in both arms will receive standard in-hospital care for severe anaemia and a 3-day course of artemether-lumefantrine at discharge. At 2 weeks after discharge surviving children will be randomized to receive either 3-day courses of dihydroartemisinin-piperaquine at 2, 6 and 10 weeks or an identical placebo and followed for 26 weeks through passive case detection. The trial will be conducted in hospitals in malaria-endemic areas in Kenya and Uganda. The study is designed to detect a 25% reduction in the incidence of all-cause re-admissions or death (composite primary outcome) from 1,152 to 864 per 1000 child years (power 80%, α=0.05) and requires 520 children per arm (1040 total children).

Results

Participant recruitment started in May 2016 and was on-going by the time this article was published.

Trial registration:

ClinicalTrials.gov NCT02671175, Registered January 28, 2016.

https://clinicaltrials.gov/ct2/show/NCT02671175

Keywords: Malaria, severe anaemia, chemoprevention, post-discharge, readmission, mortality, dihydroartemisinin-piperaquine, protocol, cost-effectiveness

Background

Severe anaemia, defined as haemoglobin (Hb) concentration level below 5.0 g/dL or haematocrit below 15.0%,[1] is a major public health problem in low and middle-income countries. Severe anaemia is associated with approximately one-third of hospital admissions among febrile children in sub-Saharan Africa contributing substantially to paediatric morbidity and mortality, especially in malaria-endemic areas.[2,3] Children under 5 years of age are most vulnerable to the long term effects of severe anaemia including decreased cognitive performance, mental and motor development.[4] Rates of in-hospital mortality due to severe anaemia ranging from 4 to 12% have been reported in different epidemiological settings.[5-7] In addition, these reports indicate high post-discharge mortality and morbidity, especially in the first 3 to 6 months. Longitudinal follow-up of children aged less than 5 years admitted with severe anaemia in Malawi showed that 8.2% died by 6 months post-discharge and 5.9% was readmitted with severe anaemia, compared to those without severe anaemia among whom 1.6% died and 0.5% were readmitted.[8,9] Similar high rates of post-discharge mortality (10% by 8 weeks) were observed in malaria-endemic areas of western Kenya[10] and in Uganda, where 12% died or were readmitted within 6 months.[11]

Standard in-hospital treatment of severe anaemia in many countries in sub-Saharan Africa consists of a blood transfusion and parenteral artesunate for severe malaria.[12] In the case of severe malarial anaemia, this is completed with a 3-day course of artemisinin-based combination therapy (ACT), usually artemether-lumefantrine. Children are often discharged with a short course of iron and folate, typically with no further scheduled follow-up. Haematological recovery from malaria-associated anaemia takes at least 6 weeks.[13,14] However, many children in these areas experience episodes of new or recrudescent malaria infections after discharge which negates the initial rise in haemoglobin achieved by blood transfusion resulting in delayed haematological recovery and potentially rebound of severe anaemia and death in some.[10,15-17] Furthermore, delayed haemolytic anaemia occurring 1 to 3 weeks after artesunate treatment of falciparum malaria has been reported in non-immune traveller,[18,19] although more recent studies show this to be rare in African children.[20]

Malaria control strategies in endemic and epidemic-prone areas include Intermittent Preventive Therapy (IPT). IPT is the administration of a full treatment course using long-acting antimalarials at pre-defined time intervals irrespective of a patient's malaria status to clear existing infections and to provide prolonged prophylaxis against new infections.[21] The World Health Organisation (WHO)

recommends IPT as a malaria control strategy in malaria-endemic areas for pregnant women (IPTp),[22,23] infants (IPTi)[24] and for children in areas with seasonal malaria transmission ('seasonal malaria chemoprevention', or SMC).[25] Currently, no control strategy specifically addresses the high-risk post-discharge period for children previously treated for severe anaemia in malaria-endemic areas. In Malawi, three months of malaria chemoprevention with three full treatment courses of artemether-lumefantrine, given in-hospital to children under five years of age admitted with severe malarial anaemia, and at one and two months post-discharge, prevented 31% of deaths or readmissions by six months post-discharge, and 30% of all-cause readmissions.[17] These results are consistent with earlier findings from The Gambia which showed that in children with severe anaemia, chemoprevention targeted during the malaria transmission season halved the rate of clinical malaria and reduced all-cause hospital readmission by 78% in one trial, and recurrence of severe anaemia by 78% in the other.[26,27] These data indicate that malaria chemoprevention in the post-discharge period may provide substantial health benefits.

We are conducting an efficacy trial in Kenya and Uganda to determine the efficacy and safety of three months of malaria chemoprevention post-discharge as a potentially cost-effective strategy to reduce all-cause readmissions and deaths in children admitted with severe anaemia. We hypothesize that by creating a prophylactic-time-window post-transfusion for malaria, more time is assured for bone-marrow recovery, resulting in a more sustained haematological recovery post-discharge.

We refer to this strategy as post-discharge malaria chemoprevention, PMC, to illustrate the similarities with SMC rather than with IPT in pregnancy as it aims to provide complete, rather than intermittent prophylaxis.

Study Design and Methods

Design overview

This will be a multi-centre, parallel-group, two-arm, placebo-controlled, individually randomized, superiority trial with 1:1 allocation ratio comparing the safety and efficacy of three courses of monthly PMC-DP or placebo post-discharge provided in addition to the standard single 3-day treatment course with artemether-lumefantrine given as part of routine in-hospital care (ClinicalTrials.gov: NCT02671175. Registered January 28, 2016). Randomisation to PMC with DP or placebo will occur at 2 weeks after enrolment, and PMC treatments will be administered at 2, 6 and

10 weeks. The primary outcome will be the number of all-cause deaths or all-cause re-admissions between 2-26 weeks after enrolment (composite outcome). The study will be conducted in Uganda and Kenya, using randomisation stratified by weight and study centre. The study will include a total of 1040 children (520 per study arm) less than 5 years of age who have been admitted for severe anaemia and have successfully completed the standard in-hospital treatment.

Primary objective

The primary objective is to determine if 3 months of post-discharge malaria chemoprevention with monthly 3-day treatment courses of DP is superior to the single 3-day treatment course with artemether-lumefantrine provided as part of standard in-hospital care in reducing all-cause readmissions and deaths by 6 months in the post-discharge management of children less than 5 years of age admitted with severe anaemia.

Secondary objectives

The secondary objectives include the determination of the safety of 3 courses of monthly DP and the cost-effectiveness of PMC-DP compared to the current standard of care.

Design Considerations

The rationale for the choice of DP for PMC

Optimal antimalarial prophylaxis with maximum compliance would be provided by a regimen that is long-acting so that administration is not required more frequently than monthly. Sulphadoxine, mefloquine, and DP have sufficiently long half-lives to be considered.[28] However, there is high-level resistance to sulphadoxine in many parts of east and southern Africa, precluding its use for this purpose in these malaria-endemic areas.[29] Both amodiaquine[30] and mefloquine are poorly tolerated; which is an important consideration when providing drugs for malaria prevention to recipients with few or no symptoms[31,32] DP is very effective, well-tolerated, and provides 4 to 5 weeks of post-treatment prophylaxis, and is therefore currently the drug of choice for use for evaluation as part of IPT and malaria chemoprevention in areas with high-grade parasite resistance to sulphadoxine.[33-38] Furthermore, recent studies show that artemether-lumefantrine and DP exert inverse selective pressure on *P. falciparum* drug sensitivity,[39] suggesting that the use of DP may be a good choice for chemoprevention in areas where artemether-lumefantrine is the first-line drug of choice for case-management.

Why in this study population?

The primary study population involves children with severe anaemia, rather than only children with severe malarial anaemia, which was the study population in the previous trial in Malawi.[17] This is based on observational studies in Malawi, Uganda and western Kenya showing that children admitted with severe anaemia appear to be at increased risk of readmission and death regardless of whether they had evidence of malaria infection at the time of admission or not [Desai et al, unpublished observations; Richard Opoka, unpublished observations].[17] Reliable diagnosis of the presence of malaria is difficult and the differentiation between severe anaemia and severe malaria anaemia is not always feasible as it is common practice in many hospitals in sub-Saharan Africa to start parenteral treatment with antimalarials before the laboratory diagnosis of malaria is available. Furthermore, the interpretation of malaria diagnostic tests on admission may be complicated in children who received antimalarial treatment just prior to admission.[40]

Efficacy and effectiveness of delivery mechanisms

This current trial is an efficacy trial and each treatment course will be provided by study staff directly. The first dose of each course will be observed, and where feasible, doses on day 2 and 3 will also be given under supervision, or compliance verified by home visits or contacting caretakers by mobile phone. A separate trial, focusing on the effectiveness of different delivery mechanisms is being conducted by our consortium members in Malawi (NCT02721420).

Why this composite primary outcome?

Use of clinical malaria as the primary outcome would require a smaller study, however, the composite outcome is used because it is more likely to drive policy. We use a composite outcome rather than a single severe outcome, such as death, to keep sample size requirements manageable.

The rationale for assessment by 6 months after enrolment

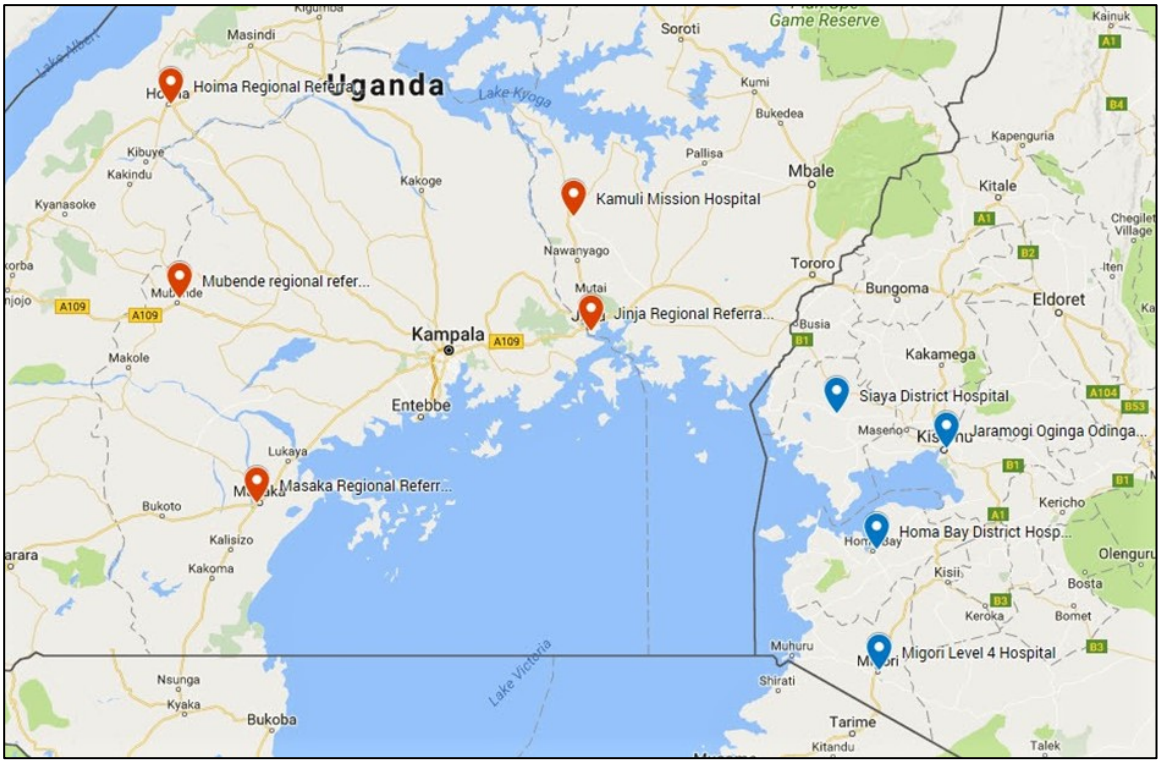
The period 2-26 weeks, instead of 0-26 weeks is used for the primary efficacy analysis because children will not be randomized until 2 weeks after enrolment. Prior to 2 weeks, all children, including those in the placebo arm, will receive a 3-day course of artemether-lumefantrine as part of standard in-hospital care, which will be started before discharge and completed at home after discharge. The duration of post-treatment prophylaxis in our previous trial with artemether-lumefantrine is about 2 to 3 weeks[17] and we, therefore, do not anticipate any differential effect between the arms until children receive their first study-specific intervention upon randomisation. The protective drug levels have waned in many children by 14 weeks (i.e. about 4 weeks after the

last PMC course of DP), but we follow the children for a total of 26 weeks to capture any potential prolonged benefits or rebound effects.

Study settings

The study will be conducted in hospitals in Kenya and Uganda located in areas with moderate to intense malaria transmission.[41,42] The annual entomological inoculation rates vary widely, in western Kenya from 31.1 to 108.6 infective bites/person/year[43,44] in areas around Kisumu and Siaya respectively, while in Uganda from 2.8 to 4 infective bites/person/year[45,46] in areas around Jinja and Mubende respectively. In western Kenya, we will recruit participants from hospitals located in areas around Lake Victoria with well-documented malaria transmission intensity, including the Jaramogi Oginga Odinga Teaching and referral hospital, Siaya, Kisumu, Homa Bay and Migori County referral hospitals. In Uganda, we will recruit from Jinja, Hoima, Masaka and Mubende regional referral hospitals as well as Kamuli mission hospital (Figure 1).

Figure 1: Map of study setting in Kenya and Uganda



Study sites in both western Kenya and Uganda are in the lake endemic region. These are large referral hospitals in the region with adequate diagnostic and treatment capacities for malaria and other conditions.

Inclusion and exclusion criteria

The inclusion and exclusion criteria for pre-study screening, enrolment and for randomisation 2 weeks later are shown below.

Eligibility criteria

Eligibility criteria for pre-study screening

Inclusion criteria for enrolment into pre-study screening period

1. Haemoglobin <5.0 g/dL or parked cell volume < 15.0%, or requirement for blood transfusion for other clinical reasons on or during admission to the hospital
2. Aged less than 59.5 months
3. Bodyweight ≥5 kg
4. Resident in the catchment area

Exclusion criteria for enrolment into pre-study screening period

1. Recognised specific other cause of severe anaemia e.g. trauma, haematological malignancy, known bleeding disorder
2. Known sickle cell disease
3. The child will reside for more than 25% of the 6 months study period (i.e. 6 weeks or more) outside of catchment area

Eligibility criteria for enrolment into the study

Inclusion criteria for enrolment:

1. Fulfilled the pre-study screening eligibility criteria
2. Aged less than 59.5 months
3. Clinically stable, able to take oral medication
4. Subject completed blood transfusion(s) or became clinically stable without transfusion
5. Able to feed (for breastfeeding children) or eat (for older children)
6. Provision of informed consent by parent or guardian

Exclusion criteria for enrolment:

1. Previous enrolment in the present study

2. Known hypersensitivity to study drug

3. Use or known need at the time of enrolment for concomitant prohibited medication including drugs known to prolong the QTc interval during the 14 weeks PMC treatment period (Additional file 1), section 8.5.7, page 34)

4. Ongoing or planned participation in another clinical trial involving ongoing or scheduled treatment with prohibited medicinal products or active follow-up during the study

5. A known need for scheduled surgery during the subsequent course of the study

6. Anticipated non-compliance with the follow-up schedule

7. Know heart conditions, or family history of congenital prolongation of the QTc interval

Eligibility criteria for randomisation into the study (at 2 weeks post-discharge)

Inclusion criteria for randomisation:

1. Fulfilled enrolment eligibility criteria and was enrolled during a recent admission

2. Aged <60 months

3. Still clinically stable, able to take oral medication, able to feed (for breastfeeding children) or eat (for older children) and able to sit unaided (for older children who were already able to do so prior to hospitalisation)

Exclusion criteria for randomisation:

1. Used DP since enrolment

2. Use or known need at the time of randomisation for concomitant prohibited medication (Additional file 1) section 8.5.7, page 34).

3. Enrolled, or known agreement to enrol into another clinical trial involving ongoing or scheduled treatment with medicinal products during the study.

4. Withdrawal of consent since enrolment

Interventions

Trial Medication and Interventions

Children will be randomized to one of the two treatment groups: DP or placebo. Children in both arms will receive standard in-hospital care and, at discharge (enrolment) a 3-day course of artemether-lumefantrine regardless of whether they were admitted with severe malarial anaemia or severe anaemia without evidence of malaria.

Artemether-lumefantrine

The study will use a Good Manufacturing Practice (GMP) formulation of artemether-lumefantrine (Coartem®, Novartis Pharmaceuticals). The recommended treatment is a 6-dose regimen over a 3-day period with dosing per bodyweight following WHO dosing recommendations as provided for in the latest WHO malaria treatment guidelines[12] (see Additional file 1, Table 3, page 29).

Dihydroartemisinin-piperaquine

The study will use the Eurartesim® brand of DP from Alfasigma (formerly Sigma Tau), Italy, a co-formulated tablet containing 40 mg dihydroartemisinin and 320 mg piperaquine phosphate or as 20/160 (paediatric formulation). Dosing will be per bodyweight according to the schedule recommended by current WHO's Guidelines (Additional file 1), Table 4, page 30).

Placebo DP

Placebos for DP will be manufactured by Alfasigma, Italy. The dosage regimen for DP-placebo will be identical in the number of tablets per day and timing of the dose to that of the active DP product. The drug administration procedures will also be identical to that for the active drugs.

Other medication

Standard in-hospital and post-discharge care

Except for the full 3-day course of artemether-lumefantrine all care provided prior to and following enrolment of the participants in the study (at convalescence) will be according to local (hospital) or national guidelines, and therefore not subject to this study. Treatment for malaria in both Kenya and Uganda conforms with the current WHO malaria treatment guidelines,[12] which includes artemether-lumefantrine as first-line treatment for uncomplicated malaria and parenteral artesunate for severe malaria. Details of non-study specific care provided by the hospital staff will be recorded.

Iron and folate supplementation

All children will receive 28 days iron and folate supplementation at 2 weeks -post-discharge as part of routine care for severe anaemia. A standardized prophylactic dose of iron supplementation (about 2 mg/kg) will be given as monotherapy or as part of the fixed-dose formulation with folic acid.

Outcomes

The Primary and secondary efficacy outcomes are shown below:

Primary efficacy outcome

The number of all-cause deaths or all-cause re-admissions between 2-26 weeks after enrolment (composite outcome).

Key secondary efficacy outcomes

1. Readmission due to severe malaria (defined as any treatment with parenteral quinine or artesunate, or presence of severe anaemia and treatment with oral antimalarials) by 26 weeks from randomisation

2. Readmissions due to severe anaemia (defined as Hb <5.0g/dL or Packed Cell Volume <15.0% or requirement for blood transfusion based on other clinical indication) by 26 weeks from randomisation

3. Readmission due to severe malarial anaemia (severe anaemia plus parenteral or oral antimalarial treatment) by 26 weeks from randomisation

4. Readmission due to severe anaemia or severe malaria (composite outcome) by 26 weeks from randomisation

5. All-cause mortality by 26 weeks from randomisation

6. All-cause hospital readmission by 26 weeks from randomisation

7. Clinic visits because of smear or malaria rapid diagnostic test (RDT)- confirmed non-severe malaria by 26 weeks from randomisation

Other secondary efficacy outcomes

1. Readmission due to severe malaria-specific anaemia (severe anaemia plus parenteral or oral antimalarial treatment and parasite density >5000/microlitre) by 26 weeks from randomisation

2. Readmission due to severe disease other than severe anaemia and severe malaria by 26 weeks from randomisation

3. Non-severe all-cause sick-child clinic visits by 26 weeks from randomisation

4. Non-malaria sick child clinic visits by 26 weeks from randomisation

5. Malaria infection at 26 weeks

6. Hb at 26 weeks

7. Any anaemia (Hb<11 g/dL), mild anaemia (Hb 8.0-10.99 g/dL) moderate anaemia (Hb 5.0-7.99 g/dL) and severe anaemia (Hb<5 g/dL) at 26 weeks

8. Weight-for-age, height-for-age, and height-for-weight Z-scores [standard deviation (SD) scores of reference population] at 26 weeks

Tolerability and safety outcomes

1. Serious adverse events, excluding primary and secondary efficacy outcomes, by 26 weeks from randomisation

2. Serious adverse events within 7 days after the start of each course of PMC, excluding primary and secondary efficacy outcomes

3. Adverse events by 26 weeks from randomisation

4. Adverse events within 7 days after the start of each course of PMC

5. QTc prolongation measured by electrocardiogram (ECG) 4-6 hours after 3rd dose of each course (in a subset of patients)

Participants timeline

Overview of study phases and scheduled visits (Table 1 and Additional file 2)

The study timelines consist of an in-patient pre-study screening period while the patient is acutely ill (visit 1), followed by a screening, consent and enrolment visit (visit 2). During the convalescence phase in the hospital, patients receive artemether-lumefantrine (visit 3), prior to discharge. The patient returns to the study clinic 14 days later (visit 4) for randomisation. Home treatment visits are made at 6 (visit 5) and 10 (visit 6) weeks. The PMC period starts at 2 weeks and ends at 14

weeks, but participants are followed up for an additional 12 weeks through passive follow-up and then seen at 26 weeks (visit 7) for an end of study assessment.

Table 1: Study Design and Schedule of Assessment (Spirit figure)

Phase	Recruitment Phase	Enrolment	In-patient Hospitalisation phase			Randomisation	PMC Treatment Phase 12 weeks period from 2-14 weeks									Post-PMC Extended follow-up Phase
Location	In-Hospital					Clinic/ Home	Home									Clinic/home
Visit number	#1	#2	#3			#4			#5			#6				#7
Visit description	Pre-study Screening	Screening Consent & Baseline	AL treatment visit			t=2 weeks; Allocation & treatment visit			t=6 weeks treatment visit			T=10 weeks treatment visit				End of study Assessment
Study Time	Days -4[a]-0	Day0	Day0 Hosp	Day1 hosp/ home	Day2 hosp/ home	2 weeks (day 14[11-28])[c]			6 weeks (day 42 [38-56])[c]			10 weeks (day 70 [66-84])[c]				6 Month (day 182 +/- 28)[c]
						2w-Day1	2w-Day2	2w-Day3	6w-Day1	6w-Day2	6w-Day3	10w-Day1	10w-Day2	10w-Day3		
Recruitment																
Pre-screening eligibility	X															
Prior consent discussion	X															
Enrolment																
Eligibility screen		X														
Informed Consent		X														
Study code issued		X														
Allocation						X										
Interventions																
PMC-Placebo arm			AL1&2[b]	AL3&4[b]	AL5&6[b]	Plac1	Plac2	Plac3	Plac1	Plac2	Plac3	Plac1	Plac2	Plac3		
PMC-Active arm			AL1&2[b]	AL3&4[b]	AL5&6[b]	DP1	DP2	DP3	DP1	DP2	DP3	DP1	DP2	DP3		
Iron supplement.						Iron for 28 days from t=14-42 days										
Assessments Baseline																
Copy Clinic/Lab data from hospital records		X				X										
Physical Exam.		X				X										
Blood sample		2ml VP				FP-Hb/MS[e]										

Efficacy Outcomes															
Physical exam/growth															X
Hb & Malaria & PCR															X
Clinic visits				Passive surveillance in clinics in the catchment area, 26 weeks from 0-26 weeks (clinical malaria and other acute illnesses) (RDT/smear, Hb, dried blood spots for parasite genetics)												
Hospitalisation				Passive surveillance for hospital admission in the catchment area, 26 weeks from 0-26 weeks											
Vital status				X	X	X	X	X	X	X	X	X	X	X	X
Pf genetics/resistance	X[f]		X				X								X
Host genetics			X												X
Patient costs						X									X
Safety Outcomes															
Adverse events				X	X	X	X	X	X	X	X	X	X	X	X
ECG[d]							X		X	X		X	X		X

Visit #1: Pre-study Screening (around admission or shortly thereafter)
Visit #2: Screening Consent & Baseline (during convalescence)
Visit #3: Oral artemether-lumefantrine (AL) consisting of 6 doses (2x daily for 3 days); first dose provided in the hospital. Subsequent doses may be administered at home or in hospital.
Visit #4: 2 weeks after enrolment. Participants will be randomised to one of the two treatment groups during this visit. They will also be given the first dose of PMC under observation. Doses of day 2 and 3 can be taken at home. All participants will get a 1-month supply of iron during this visit.
Visit #5 #6: Home visits at 6 and 10 weeks after enrolment to issue participants with the 2nd and 3rd course of the PMC study drugs.
Visit #7: at 6 months after enrolment. This is the closeout assessment.

a. Children can be pre-study screened at any time between hospital admission and enrolment. The figure of -4 days is provided for illustration purposes only.
b. AL: Some children may have received AL as part of standard in-hospital care prior to enrolment (e.g. during days -1 or -2 and not as part of the study). They will have their number of study AL doses adjusted to ensure that no more than a cumulative total of 6 AL doses are provided. The day of enrolment is always considered as Day-0 regardless of when the first dose of AL was received.
c. Visit window= number of days an actual subject visit may fall outside of the planned protocol schedule visit to still meet protocol requirements. DP should be given at least 4 weeks apart.
d. ECG, Electrocardiogram, to be conducted in a sub-sample only. A capillary sample will be taken at the same time as the ECG for piperaquine drug levels.
e. MS, malaria smear. This will be collected for research purposes only and read days to weeks later. Malaria smears will not be used for point of care. If participants are symptomatic (e.g. fever) an RDT will be taken for point of care.
f. Uses leftover samples from blood-group typing and cross-matching or other clinical samples that were taken as part of routine care that would otherwise be discarded. The sample will only be used after consent has been obtained in the subsequent visit 2.
VP=vena puncture. FP=finger prick, Plac=Placebo DP, DP=dihydroartemisinin-piperaquine, AL=artemether-lumefantrine, Hb=haemoglobin, MS=malaria smear, Pf=Plasmodium falciparum

Unscheduled Visits (passive follow-up)

A passive surveillance system is in place to monitor intercurrent illnesses during the observation period. Parents are instructed to bring their child to the study clinic for any suspected illness. Blood samples for Hb, malaria diagnosis (RDT and smear) and filter paper dry blood spot (DBS) for parasite genetics are obtained. Verbal autopsy is conducted for children who die at home during the follow-up period. Adverse events and vital status are assessed during all scheduled or unscheduled visits.

Sample Size

Original sample size

The initial estimate of the required sample size of 2212 children (1106 per arm) across both countries pooled was designed to detect a 30% reduction in the incidence rate of the composite primary outcome (death or all-cause readmission) from 469 per 1000 child years in the control arm to 328 per 1000 child years in the intervention arm (power 90%, α=0.05), which allowed for one interim analysis and 15.7% loss to follow-up. For these estimations, we assumed an average pooled event rate of 399 per 1000 child years across the two arms, with 328 and 469 events per 1000 child years in the intervention and control arms, respectively (RR=0.70). We based this assumption on observations in western Kenya (Desai et al, unpublished) and Malawi.[17] However, the observed event rate, pooled across both arms, during the first year of the study was 1,120/1000 child years, which is almost three times higher than the assumed event rates. The higher rate is consistent with the recently published observations in Uganda.[11] Furthermore, the observed rate of loss to follow-up in the first 533 participants recruited and followed up for 6 months was 7% rather than the assumed 15%.

Sample size re-estimation

Following recommendations from the Data Monitoring and Ethics Committee (DMEC) and The Trial Steering Committee (TSC), a blinded interim sample size re-estimation was conducted to take into account the lower than expected rate of loss to follow-up and the higher than expected pooled incidence rate of the composite primary endpoint (death or all-cause readmission). This was favoured over an interim analysis because the available funding did not allow an extension of the recruitment period, even if the results of any interim analysis had suggested that this would be required.

The revised sample size calculations show that a total sample size of 1040 children (520 per arm) is required to detect a 25% reduction in the incidence of the composite primary outcome from 1,152 per 1000 child years (530 events per 1000 children) in the control arm to 864 per 1000 child years (398 per 1000 children) in the intervention arm (power 80%, α=0.05), allowing for 10% loss to follow-up. The same sample size also provides 90% power to detect a 28.7% reduction in the primary endpoint from 1,152 to 822 events per 1000 children years.

Assignment of interventions

Allocation

Eligible children are randomly assigned (1:1) to either PMC-DP or placebo by a computer-generated randomisation schedule stratified by weight (per DP dosing schedule) and study site using permuted blocks of random sizes (see Additional file 1, page 30, Table 4)[12] Recruitment is 'competitive' between the sites in the trial.

Blinding

The study is double-blinded to both participants/caretakers and study staff. Allocation concealment is achieved by the use of sealed opaque envelopes, with each envelope containing 3 other small envelopes (one for each PMC course). The envelopes containing active DP or placebo look identical, and the appearance and consistency of the tablets are also identical.

Laboratory Procedures

Hb is measured using HemoCue201 (Angelholm, Sweden) photometers. Thick and thin blood films for parasite counts is obtained and examined. The films are read by two independent microscopists by counting any malaria parasites against 200 high power fields before a slide is declared negative.[47] Point of care malaria diagnosis will be conducted using First Response® Malaria Ag. pLDH/HRP2 Combo Card Test

Statistical Methods

A detailed study statistical analytical plan for the final analysis, that will supersede the study protocol, will be developed during the study before the unblinding of data.

Analysis Populations

The intention-to-treat population (ITT) is defined as all randomized subjects allocated to one of the two treatment arms and will be analysed in the group to which they were randomized, regardless

of the type (placebo or active PMC) or the number of courses received. The per-protocol (PP) population is a subset of the ITT population; excluding participants with major protocol deviations.

Missing Data

Every effort is being made to minimise the amount of missing data in the trial, and whenever possible, information on the reason for missing data is obtained. No adjustments will be made for missing outcome data, but missing data may be imputed for co-variates.

Assessment of efficacy

The primary analysis will be by intention to treat and include all primary endpoint events (i.e. first and repeat events). The follow-up time will be measured as the time in days from the date of randomisation to the end of follow-up (around 26 weeks), death or drop-out. The incidence rate will be calculated per arm and the incidence rate ratio (IRR, PMC to placebo) and 95% CI estimated using Poisson regression models with treatment (as randomised) as the only covariate. The results will also be expressed as the relative rate reduction (RRR) (95% CI). (However, in the final analysis, we used cox regression for repeated events as this takes time to each event into account. The results were expressed as hazard ratio (RR) and 95% confidence interval).

Subgroup analysis

We will use stratified analysis to assess to what extent the effect of the intervention on the primary outcome is influenced by country, demographic parameters (e.g. age, ethnicity and socioeconomic status), clinical parameters, malaria transmission variables (malaria transmission intensity, residence (urban/rural), season, insecticide-treated nets use, site), time of assessment and potential intervention modifiers. Because we did not power the study for subgroup analyses, we will interpret the results of the subgroup analysis cautiously. No adjustment will be made for multiple comparisons.

Sensitivity analysis

A number of sensitivity analyses will be conducted to assess the robustness of the primary endpoint analysis. These include analysis of the per-protocol subject population and covariate-adjusted analysis. Other regression models will also be explored. Additional post-hoc analyses may also be conducted if deemed appropriate. In addition, we will compare the results of the covariate-adjusted analyses with and without imputation for missing values for co-variates values at baseline.

Analysis of adverse events

Adverse events and serious adverse events are monitored, managed, and recorded during the study. They will be recorded and tabulated for each treatment arm, overall, and per body system. Treatment-emergent adverse events are defined as adverse events that had an onset day on or after the day of the first dose of study medication. No formal statistical testing will be undertaken. Enrolled children who are clinically unstable 2 weeks post-discharge (i.e. at the time eligibility is assessed for randomization) and/or have rebound severe anaemia, are re-admitted and become eligible for randomization if they fulfil the entry criteria two weeks after the subsequent discharge.

Procedures for Assessing Efficacy and safety Parameters

Primary Efficacy outcome

All-cause Mortality

This will be assessed during visits 4 (2 weeks), 5 (6 weeks), 6 (10 weeks), and 18 weeks (by phone) and during the end of study assessment at 26 weeks.

All-cause and Disease-Specific Re-admissions

This will be assessed through passive case detection and through a questionnaire administered during visits 4-7 at 2, 6, 10, 26 weeks and during unscheduled sick visits. Details of admissions and treatment that the participants received are recorded, including malaria diagnostic test results and use of antimalarials to allow for differentiation between malaria, severe anaemia, and other syndromes.

Secondary Efficacy Outcomes

All-Cause and malaria-specific Clinic Visits

This will be assessed through passive case detection and through questionnaires administered during visits 4-7 at 2, 6, 10, 26 weeks and during unscheduled sick visits. Details of clinic visits are recorded including malaria diagnosis results to allow for differentiation between malaria and non-malaria clinic visits.

Adverse Events

We will adhere to the International Conference on Harmonisation (ICH) good clinical practice (GCP) principles in recording, reporting and managing adverse events and serious adverse events for all participants in both arms (see Additional file 1, page 51, section 9.6.2).

Cardiac monitoring sub-study

The main safety concern with DP is its dose dependent QTc prolongation induced by the piperaquine component. Transient QTc prolongation has been confirmed in clinical trials but there are no data suggesting that the treatment is associated with clinically significant arrhythmias.[38,48,49] A trial in Uganda among children 6 to 24 months old included monthly DP for up to 18 monthly courses. A detailed sub-study of the effect of DP on cardiac repolarization was conducted in 26 children and concluded that DP is not associated with a trend toward increasing QTc prolongation with an increasing number of DP courses.[38] This type of safety data is limited and we will, therefore, conduct a nested cardiac monitoring sub-study at Jinja Regional Referral Hospital in Uganda among 66 children who will be selected through convenience sampling. Separate written informed consent will be sought for inclusion in this sub-study. Approximately half of these children are expected to have received PMC with DP. The primary objective is to determine whether transient QTc prolongation increases in magnitude with subsequent courses of DP. Children enrolled in the sub-study will have an ECG taken prior to the first dose of each course and again 4-6 hours after taking the 3rd dose of each course of DP (anticipated maximum drug concentration).

Discussions

Severe anaemia and severe malaria are major public health problems in malaria-endemic areas of Africa. Evidence suggests that a major, potentially preventable, component of the burden occurs after discharge and that a proactive approach is needed. Currently, no strategy specifically addresses this high-risk post-discharge period. This study seeks to determine the efficacy, safety and cost-effectiveness of 3 months of malaria chemoprevention post-discharge as an innovative strategy to reduce all-cause readmissions and deaths among children admitted with severe anaemia in malaria-endemic areas. The study settings in Kenya and Uganda are representative of the main epidemiological settings appropriate for this intervention. Members from our consortium, under the leadership of the College of Medicine in Malawi, are concurrently conducting a trial in Malawi, under a separate protocol, on potential delivery mechanisms and health services research to determine the uptake, effectiveness, acceptability and feasibility of different mechanisms for delivering PMC (clinicaltrials.gov: NCT02721420). This strategy builds on existing approaches used for seasonal malaria chemo-prevention in west Africa and experience with IPT in pregnant women and infants [50,51]. Should PMC prove to be effective, cost-effective and feasible, PMC may be a

promising strategy to reduce all-cause readmissions and deaths in children admitted with severe anaemia in malaria-endemic areas of Africa.

Trial status

Recruitment started in May 2016 and is ongoing. Unblinding and analysis will begin after recruitment and follow up is completed, the database has been completed, cleaned, and locked.

Additional files

Additional file 1: Full study protocol (including SPIRIT figure): v4.0, dated 06-Feb-2018.

Additional file 2: Summary of study design and schedule of assessment (SPIRIT checklist)

Additional file 3: Ethics Approvals KEMRI, SOMREC, LSTM, REK vest and CDC

All additional files are available online at https://trialsjournal.biomedcentral.com/articles/10.1186/s13063-018-2972-1

Declarations

Ethical considerations

This protocol, the informed consent documents and patient information sheets have been reviewed and approved by KEMRI Scientific and Ethics Review Unit (SERU) (protocol #2965), Makerere University School of Medicine Research and Ethics Committee (SOMREC) (protocol #2015-125), LSTM Research Ethics Committee (protocol #14.034), Regional Committee for Medical and Health Research Ethics, Western Norway (REK Vest) (protocol #2014/1911). The Centers for Disease Control and Prevention gave approval for reliance on the KEMRI SERU (CDC Protocol #6919) (see Additional file 3, Ethics Approvals KEMRI, SOMREC, LSTM, REK vest and CDC).

Consent for publication

Not applicable

Availability of data and material

Not applicable

Oversight

The study has a trial steering committee (TSC) and a Data Monitoring and Ethics Committee (DMEC).

Acknowledgements

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Conflicts of Interest

None declared

Author contributions

FOtK and KP conceived the study. RI, RO, CJ and FtK drafted the protocol. BR, RI, RO, CJ, MD, SK, MBvH, TKK, AD, FOtK, KP further developed the study design during a protocol workshop. DW provided statistical expertise in clinical trial design. All authors contributed to the refinement of the

initial study protocol. TKK, AD and FOtK drafted the amendments and all authors contributed to the refinement the amended versions. TKK and FOtK drafted the manuscript. All authors read and approved the final manuscript prior to submission.

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Chapter 6: Post-Discharge Malaria Chemoprevention Safety and Efficacy

Monthly Malaria Chemoprevention for The Post-Discharge Management of Severe Anaemia in Children

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Manuscript Status: To be submitted for publication

Abstract

Background

Children hospitalized with severe anaemia in malaria-endemic areas are at high risk of readmission or death within six months post-discharge. No strategy specifically addresses this post-discharge period. We conducted a multi-centre, two-arm, placebo-controlled, randomised trial in nine hospitals in Kenya and Uganda to determine if three months of post-discharge malaria chemoprevention (PMC) with monthly 3-day treatment courses of dihydroartemisinin-piperaquine (DP) reduced the rate of all-cause readmissions and deaths by 6 months post-discharge (primary outcome).

Methods

Children aged <5 years with admission haemoglobin of <5g/dL were eligible. They received standard in-hospital care for severe anaemia and a standard 3-day course with artemether-lumefantrine at discharge. At two weeks post-discharge, they were randomised to receive DP or placebo-DP at two, six, and ten weeks post-discharge and followed until week 26 inclusive using passive case-detection. Conditional risk set modelling for repeated events (Prentice-Williams-Peterson total-time) was used to obtain hazard ratios (HR).

Results

Between May 2016 and November 2018, 1049 participants were randomised (PMC=524, control=525). Between 2-26 weeks post-discharge, there were 184 primary outcome events in the PMC arm and 316 in the placebo arm (Hazard Ratio [HR]=0.65, 95% CI 0.54-0.78). The HR was 0.30 (0.22-0.42) during the PMC-intervention period (2-14 weeks) and 1.13 (0.87-1.47) during the post-intervention period (15-26 weeks), p<0.001). The median time to the first event was delayed from 47 to 110 days.

Conclusion

In areas with intense malaria transmission, three months of malaria chemoprevention in children recently admitted with severe anaemia results in major reductions in all-cause readmissions or death post-discharge.

Introduction

Severe anaemia is a substantial contributor to child mortality in malaria-endemic Africa. A third of children aged <5 years admitted with fever are severely anaemic comprising 4 to 12% of in-hospital mortality.[1,2]

In the past 2-3 decades, most research on severe anaemia in Africa focused on reducing in-hospital mortality. However, observational studies show that in areas with intense malaria transmission a significant, potentially preventable component of the burden of severe anaemia occurs in the first few months post-discharge with similar or higher mortality than during the in-hospital period.[3,4] These observations suggest that strategies that reduce the risk in this post-discharge period could offer substantial public health gains.

Data from Malawi show that children in malaria-endemic areas with severe anaemia require at least two to three months to achieve haematological recovery.[3] In areas with intense malaria transmission, delay in haematological recovery due to new or recrudesced malaria infections is common and may contribute to the high post-discharge burden.[4,5] We hypothesized that by creating a prophylactic-time-window post-transfusion, the bone marrow gets time to recover, resulting in a more sustained haematological recovery post-discharge. Currently, there are no specific prevention strategies provided post-discharge to address this in malaria-endemic areas. The World Health Organization (WHO) recommends seasonal malaria chemoprevention (SMC) with monthly treatment courses of antimalarials to reduce the burden of malaria during the transmission season,[6] and intermittent preventive therapy (IPT) for pregnant women (IPTp).[5] In Malawi, three months of post-discharge malaria chemoprevention (PMC) with monthly treatment courses of artemether-lumefantrine prevented 31% of deaths or readmissions by six months post-discharge in children aged <5 years who had been admitted with severe malarial anaemia and were successfully treated with blood transfusion and parenteral antimalarials.[7]

In this study, we aimed to establish the safety and efficacy of 3 months of post-discharge malaria chemoprevention with monthly 3-day treatment courses of the long-acting antimalarial dihydroartemisinin-piperaquine (DP) in reducing all-cause readmissions and deaths by six months in the post-discharge management of children aged <5 years admitted with all-cause severe anaemia.

Methods

Design and Oversight

We did a randomised, double-blind, placebo-controlled, parallel two-arm superiority trial. The trial was conducted in nine hospitals in Kenya and Uganda located in areas with moderate to intense perennial malaria transmission.[8] It was approved by the ethics committees of the Kenya Medical Research Institute (KEMRI), the Makerere University School of Medicine, the Regional Committee for Medical and Health Research Ethics, Western Norway and the Liverpool School of Tropical Medicine (LSTM). Dihydroartemisinin-piperaquine and placebo were supplied by Alfasigma (formerly Sigma-Tau), Italy which had no role in the design of the trial; the collection, analysis, or interpretation of the data or the writing of the manuscript. Trial oversight and monitoring were provided by a trial steering committee and by an independent data and safety monitoring committee, who archived a signed copy of the statistical analysis plan before the database was locked and the data were unmasked. The trial methods were published previously.[8] Additional details are provided in the Supplementary Appendix and the protocol and statistical analysis plan will be available at nejm.org after publication. The authors TK, VW, DW and FOtK vouch for the accuracy and completeness of the data and analyses and all authors vouch for the fidelity of the trial to the protocol.

Randomisation and masking

Children who had been admitted with severe anaemia and fulfilled the other eligibility criteria (Supplementary Appendix) were randomly assigned (1:1) 2 weeks after discharge to receive either PMC or placebo using a computer-generated randomisation schedule stratified by study site and 5 weight bands (Table S2) using permuted blocks of random sizes generated by an independent statistician.

Allocation concealment was achieved using sequentially numbered sealed identical-looking opaque envelopes prepared by the independent study pharmacist. Each envelope contained three other envelopes containing the study drug for each PMC course. Participants were enrolled and followed up by study staff. Throughout the trial, the investigators, caretakers, and study staff were unaware of the trial-group assignments.

Interventions

Participants received standard in-hospital care for severe anaemia and any other conditions based on the Kenya/Uganda Ministry of Health treatment guidelines and a 3-day course of artemether-lumefantrine at discharge, irrespective of their malaria status on admission, as soon as they were able to take oral medication. Two weeks later, surviving participants were randomised to receive either standard 3-day courses of DP (Eurartesim®, Sigma-Tau, Italy) or placebo, at two, six, and ten weeks after enrolment. Participants also received a 28-day course of iron supplementation at randomisation as per the national treatment guidelines in each country.[9,10] and were encouraged to sleep under an insecticide-treated net. The artemether-lumefantrine and DP doses were based on the current WHO guidelines (Table S1 and Table S2). The first dose of each course was administered by study staff in the hospital or during home visits and the remaining doses by the guardians at home. Adherence to the study medication was ascertained by daily telephone contact with caretakers and random home visits.

Outcomes

All participants were followed passively for a total of 24 weeks after randomisation (i.e., 26 weeks after discharge; Figure S1). The primary outcome was a composite of all-cause mortality or all-cause readmission. Key secondary outcomes included the individual components of the primary outcome; hospital readmissions due to severe anaemia or malaria; and all-cause and malaria-related out-patient clinic visits (see Supplementary Appendix for additional efficacy outcomes). Tolerability and safety outcomes included adverse events reported by study clinicians and QTc prolongation measured by electrocardiogram (ECG) 4-6 hours after the third dose of each course.

Statistical Analysis

The initial sample size was 2212 children (1106 per arm) across both countries pooled. For these estimations, we assumed an average pooled event rate of 399 per 1000 child years across the two arms. We based this assumption on observations in western Kenya (Desai et al, unpublished) and Malawi.[7] However, the observed event rate, pooled across both arms, during the first year of the study was 1,120/1000 child years. The higher rate is consistent with the recently published observations in Uganda.[11] Furthermore, the observed rate of loss to follow-up in the first 533 participants recruited and followed up for 6 months was 7% rather than the assumed 15%.

Following recommendations from the Data Monitoring and Ethics Committee (DMEC) and The Trial Steering Committee (TSC), a blinded interim sample size re-estimation was conducted to take into account the lower than expected rate of loss to follow-up and the higher than expected pooled incidence rate of the composite primary endpoint.

The revised sample size calculations show that a total of 1,040 (520 per arm) participants are required to detect a 25% reduction in the incidence of the composite primary outcome from 1,152 to 864 per 1000 child years with 80% power and a two-sided p-value of 0.05 allowing for 10% loss to follow-up.[8]

Analyses were performed using STATA, version 15.1 (StataCorp). The primary outcome was analyzed as recurrent time-to-event data using the Prentice Williams Peterson-Total Time (PWP-TT),[12] and results expressed as the hazard ratio (HR), 95% confidence interval (CI) and p-value (based on the z-score). Models included site and weight category as covariates to adjust for stratification factors. The primary analysis was based on the intention-to-treat population (ITT), defined as all participants randomised and included the primary outcome events occurring during 24 weeks starting from the day of randomisation (14 days after enrolment) until the end of week-26 (day 181 inclusive), henceforth referred to as week 3-26. The analysis was also stratified *a priori* by the PMC-intervention period (weeks 3-14) and the post-intervention period when the direct pharmacological protective effect of DP has waned (weeks 15-26).

Supportive analyses using covariate-adjusted analysis (Supplementary Appendix) and per-protocol analysis were also performed. Further sensitivity analyses to assess the robustness of the primary analysis were conducted using the negative binomial model and Prentice Williams Peterson–Gap Time (PWP-GT) model.[12] Similar analyses were used for secondary time-to-event outcomes. The Kaplan-Meier survival plots were drawn for time to first event and forest plots to present the results of the PWP-TT models for primary and secondary outcomes and subgroup analyses. The p-values for the interaction terms were obtained using the Altman and Bland method.[13]

Results

Trial population

During the in-hospital period, 1,366 participants were screened, and 1,125 assessed for eligibility of whom 1,049 were randomised and included in the ITT population (PMC=524, Placebo=525, Figure

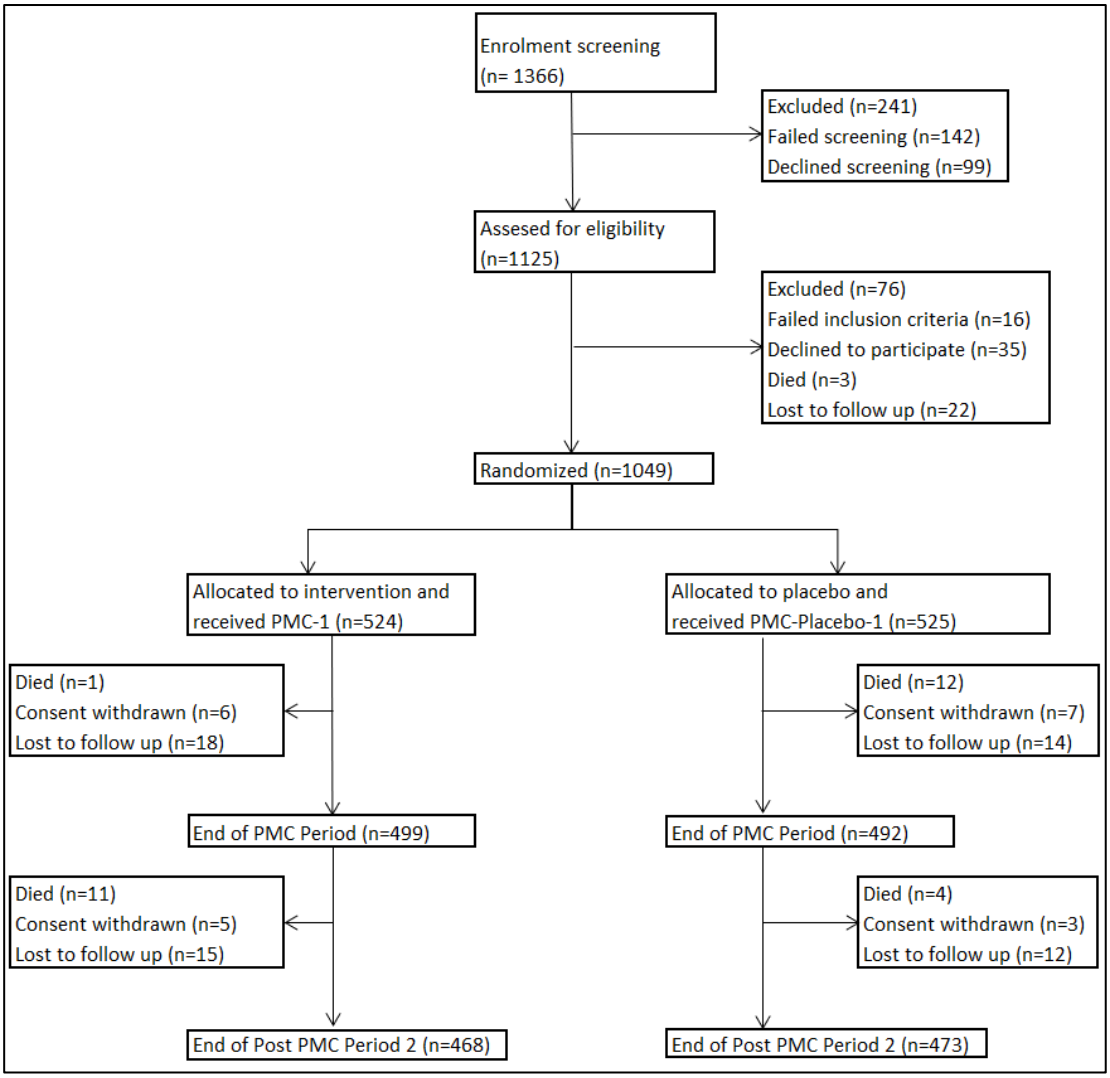
1). The baseline characteristics were similar between groups (Table 1). Overall, 96.8% of 1,041 participants received all three intended courses or had died before the third course, and 7.6% (80/1049) withdrew or were lost-to-follow-up. This was equally divided between the study arms. The median (IQR) follow-up time was 168 days (161-168) (Table S 4).

Primary outcome

Overall, 315 (30.0%) of 1,049 participants had 500 primary events: 184 and 316 in the PMC and placebo groups, respectively (Figure 2). PMC was associated with a 35% lower hazard of the primary outcome by 26 weeks (HR=0.65, 95% CI 0.54-0.78, p<0.001), and this was 70% during the PMC-intervention period from 3-14 weeks post-discharge (HR=0.30, 0.22-0.42, p<0.001), and -13% during the post-intervention period (15-26 weeks) (HR=1.13, 0.87-1.47, p=0.37), (p-value interaction term <0.001 [Figure S2]). Similar results were obtained with co-variate adjusted analysis, sub-group analysis (Figure 2 and Figure S3), per-protocol analysis (Figure S4), and sensitivity analysis using alternative extended Cox-regression models or negative binomial regression models (Figure S5).

The PMC intervention delayed the median time to first event from 47 (IQR=20-71) to 110 (IQR=53-136) days (HR=0.58, 0.47-0.73, Log-rank p=0.007) (Figure 4).

Figure 1: CONSORT diagram



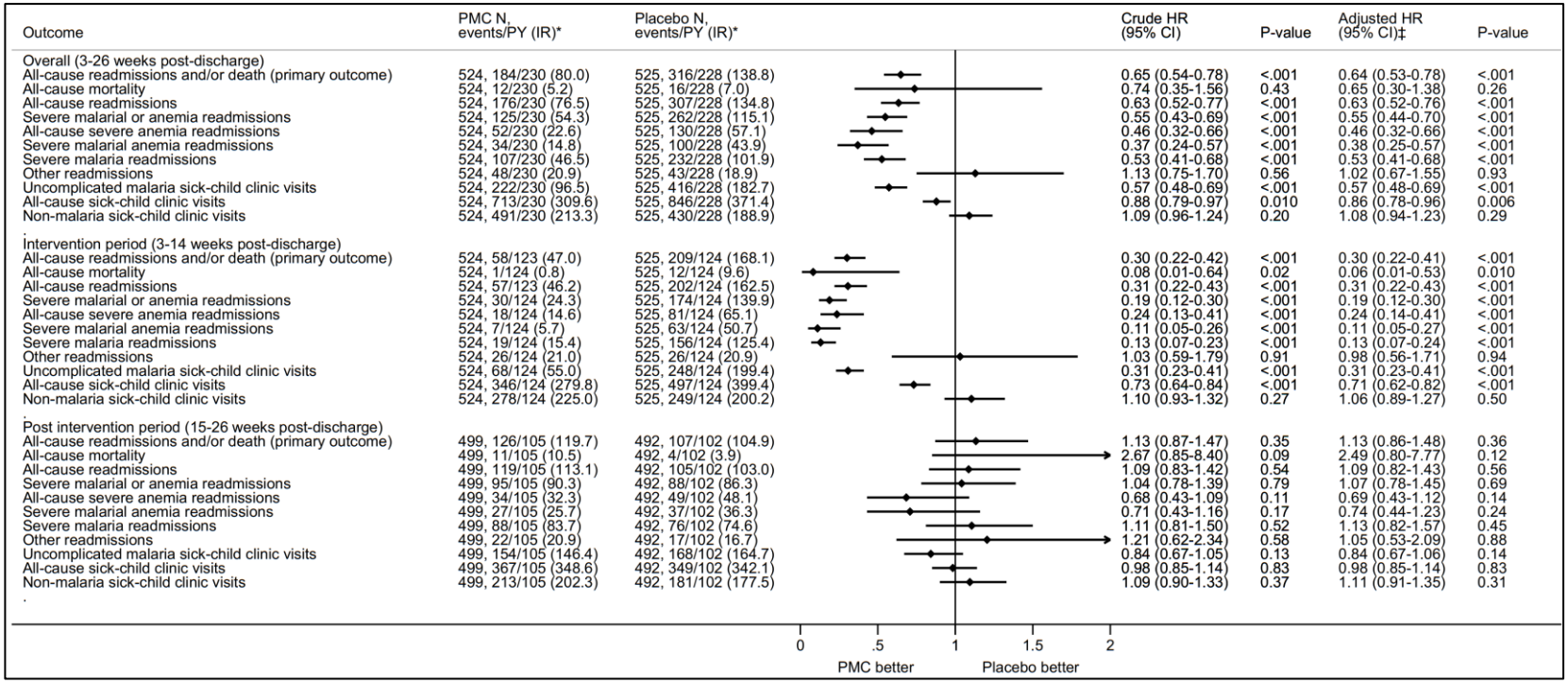
PMC, post-discharge malaria chemoprevention.

*Table 1: Baseline Characteristics of Study Participants. **

	PMC		Placebo	
Child characteristics				
Study site				
Uganda—no./total no. (%)	347/524	(66.2)	345/525	(65.7)
Kenya	177/524	(33.8)	180/525	(34.3)
Sex (male)—no./total no. (%)	280/524	(53.4)	265/525	(50.5)
Mean age in months	26.8±15.5		26.3±14.8	
Distance (km) to clinic	27.5±22.6		29.1±23.8	
SES—no./total no. (%)				
poor	173/524	(33.0)	177/525	(33.7)
poorer	164/524	(31.3)	189/525	(33.0)
poorest	187/524	(35.7)	159/525	(30.3)
Mean Hb Level (g/dL) before transfusion	4.1±1.1		4.2±1.2	
Mean Hb level (g/dL) at baseline	8.1±4.5		8.0±2.0	
Mean weight (Kg) at randomisation	11.1±3.2		11.0±3.1	
SMA—no./total no. (%)	436/524	(83.2)	454/525	(86.5)
Non-SMA—no./total no. (%)	74/524	(14.1)	61/525	(11.6)
Number of previous hospitalizations—no./total no. (%)				
1	339/521	(65.1)	330/522	(63.2)
2	94/521	(18.0)	98/522	(18.8)
>=3	88/521	(16.9)	94/522	(18.0)
Parent characteristics				
Marital Status—no./total no. (%)				
Single	41/524	(7.8)	29/525	(5.5)
Married	425/524	(81.1)	435/525	(82.9)
Other	58/524	(11.1)	61/525	(11.6)
Father unemployed—no./total no. (%)	32/134	(23.9)	22/117	(18.8)
Mother education level—no./total no. (%)				
None	36/349	(10.3)	33/366	(9.0)
Completed primary	224/349	(64.2)	248/366	(67.8)
Completed secondary	84/349	(24.1)	72/366	(19.7)
Tertiary/University	5/349	(1.4)	13/366	(3.6)

*Plus-minus values are means ±SD. PMC, post-discharge malaria chemoprevention; SES=socio economic status, RDT=rapid diagnostic test, HIV=human immune deficiency virus, SMA=severe malaria anaemia. There were no significant differences between the study groups in any characteristic. Percentages may not total to 100 because of rounding.

Figure-2: Primary and other efficacy outcomes



Outcome	PMC N, events/PY (IR)*	Placebo N, events/PY (IR)*	Crude HR (95% CI)	P-value	Adjusted HR (95% CI)‡	P-value
Overall (3-26 weeks post-discharge)						
All-cause readmissions and/or death (primary outcome)	524, 184/230 (80.0)	525, 316/228 (138.8)	0.65 (0.54-0.78)	<.001	0.64 (0.53-0.78)	<.001
All-cause mortality	524, 12/230 (5.2)	525, 16/228 (7.0)	0.74 (0.35-1.56)	0.43	0.65 (0.30-1.38)	0.26
All-cause readmissions	524, 176/230 (76.5)	525, 307/228 (134.8)	0.63 (0.52-0.77)	<.001	0.63 (0.52-0.76)	<.001
Severe malarial or anemia readmissions	524, 125/230 (54.3)	525, 262/228 (115.1)	0.55 (0.43-0.69)	<.001	0.55 (0.44-0.70)	<.001
All-cause severe anemia readmissions	524, 52/230 (22.6)	525, 130/228 (57.1)	0.46 (0.32-0.66)	<.001	0.46 (0.32-0.66)	<.001
Severe malarial anemia readmissions	524, 34/230 (14.8)	525, 100/228 (43.9)	0.37 (0.24-0.57)	<.001	0.38 (0.25-0.57)	<.001
Severe malaria readmissions	524, 107/230 (46.5)	525, 232/228 (101.9)	0.53 (0.41-0.68)	<.001	0.53 (0.41-0.68)	<.001
Other readmissions	524, 48/230 (20.9)	525, 43/228 (18.9)	1.13 (0.75-1.70)	0.56	1.02 (0.67-1.55)	0.93
Uncomplicated malaria sick-child clinic visits	524, 222/230 (96.5)	525, 416/228 (182.7)	0.57 (0.48-0.69)	<.001	0.57 (0.48-0.69)	<.001
All-cause sick-child clinic visits	524, 713/230 (309.6)	525, 846/228 (371.4)	0.88 (0.79-0.97)	0.010	0.86 (0.78-0.96)	0.006
Non-malaria sick-child clinic visits	524, 491/230 (213.3)	525, 430/228 (188.9)	1.09 (0.96-1.24)	0.20	1.08 (0.94-1.23)	0.29
Intervention period (3-14 weeks post-discharge)						
All-cause readmissions and/or death (primary outcome)	524, 58/123 (47.0)	525, 209/124 (168.1)	0.30 (0.22-0.42)	<.001	0.30 (0.22-0.41)	<.001
All-cause mortality	524, 1/124 (0.8)	525, 12/124 (9.6)	0.08 (0.01-0.64)	0.02	0.06 (0.01-0.53)	0.010
All-cause readmissions	524, 57/123 (46.2)	525, 202/124 (162.5)	0.31 (0.22-0.43)	<.001	0.31 (0.22-0.43)	<.001
Severe malarial or anemia readmissions	524, 30/124 (24.3)	525, 174/124 (139.9)	0.19 (0.12-0.30)	<.001	0.19 (0.12-0.30)	<.001
All-cause severe anemia readmissions	524, 18/124 (14.6)	525, 81/124 (65.1)	0.24 (0.13-0.41)	<.001	0.24 (0.14-0.41)	<.001
Severe malarial anemia readmissions	524, 7/124 (5.7)	525, 63/124 (50.7)	0.11 (0.05-0.26)	<.001	0.11 (0.05-0.27)	<.001
Severe malaria readmissions	524, 19/124 (15.4)	525, 156/124 (125.4)	0.13 (0.07-0.23)	<.001	0.13 (0.07-0.24)	<.001
Other readmissions	524, 26/124 (21.0)	525, 26/124 (20.9)	1.03 (0.59-1.79)	0.91	0.98 (0.56-1.71)	0.94
Uncomplicated malaria sick-child clinic visits	524, 68/124 (55.0)	525, 248/124 (199.4)	0.31 (0.23-0.41)	<.001	0.31 (0.23-0.41)	<.001
All-cause sick-child clinic visits	524, 346/124 (279.8)	525, 497/124 (399.4)	0.73 (0.64-0.84)	<.001	0.71 (0.62-0.82)	<.001
Non-malaria sick-child clinic visits	524, 278/124 (225.0)	525, 249/124 (200.2)	1.10 (0.93-1.32)	0.27	1.06 (0.89-1.27)	0.50
Post intervention period (15-26 weeks post-discharge)						
All-cause readmissions and/or death (primary outcome)	499, 126/105 (119.7)	492, 107/102 (104.9)	1.13 (0.87-1.47)	0.35	1.13 (0.86-1.48)	0.36
All-cause mortality	499, 11/105 (10.5)	492, 4/102 (3.9)	2.67 (0.85-8.40)	0.09	2.49 (0.80-7.77)	0.12
All-cause readmissions	499, 119/105 (113.1)	492, 105/102 (103.0)	1.09 (0.83-1.42)	0.54	1.09 (0.82-1.43)	0.56
Severe malarial or anemia readmissions	499, 95/105 (90.3)	492, 88/102 (86.3)	1.04 (0.78-1.39)	0.79	1.07 (0.78-1.45)	0.69
All-cause severe anemia readmissions	499, 34/105 (32.3)	492, 49/102 (48.1)	0.68 (0.43-1.09)	0.11	0.69 (0.43-1.12)	0.14
Severe malarial anemia readmissions	499, 27/105 (25.7)	492, 37/102 (36.3)	0.71 (0.43-1.16)	0.17	0.74 (0.44-1.23)	0.24
Severe malaria readmissions	499, 88/105 (83.7)	492, 76/102 (74.6)	1.11 (0.81-1.50)	0.52	1.13 (0.82-1.57)	0.45
Other readmissions	499, 22/105 (20.9)	492, 17/102 (16.7)	1.21 (0.62-2.34)	0.58	1.05 (0.53-2.09)	0.88
Uncomplicated malaria sick-child clinic visits	499, 154/105 (146.4)	492, 168/102 (164.7)	0.84 (0.67-1.05)	0.13	0.84 (0.67-1.06)	0.14
All-cause sick-child clinic visits	499, 367/105 (348.6)	492, 349/102 (342.1)	0.98 (0.85-1.14)	0.83	0.98 (0.85-1.14)	0.83
Non-malaria sick-child clinic visits	499, 213/105 (202.3)	492, 181/102 (177.5)	1.09 (0.90-1.33)	0.37	1.11 (0.91-1.35)	0.31

* N=total number contributing per time period, PY=person years, IR=Incidence rate per 100 person-years.

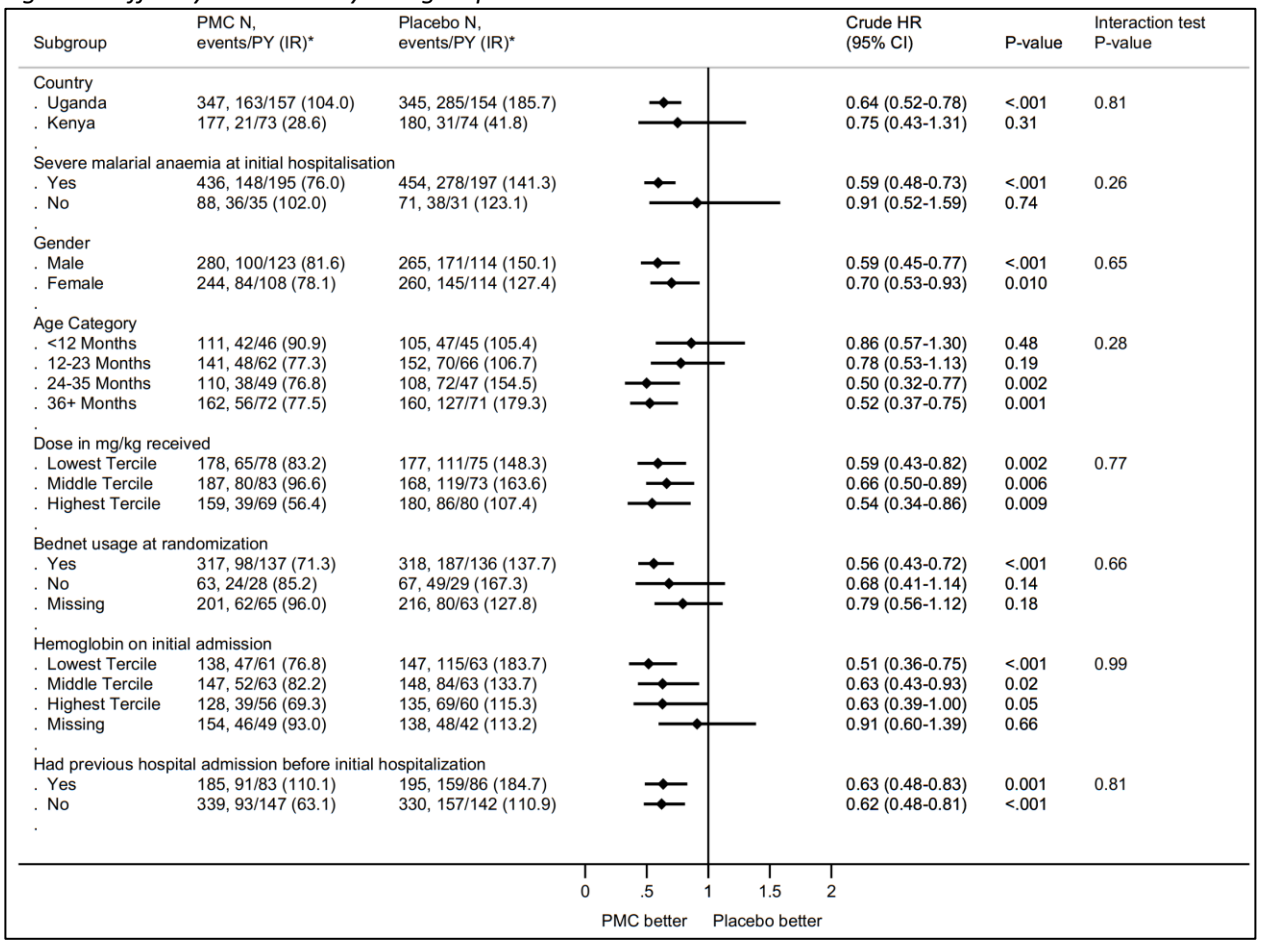
† Median and interquartile range (IQR) of the time to any event (i.e., first, second, third event, etc. pooled).

‡ Adjusted for site, bodyweight, previous hospital admissions, the syndrome on admissions, age, haemoglobin level at randomization, distance to hospital, number of previous admissions, and socio-economic status.

PMC=Post-discharge malaria chemoprevention

Confidence intervals for secondary outcomes have not been adjusted for multiple testing, and inferences drawn from the intervals may not be reproducible.

Figure-3: Efficacy outcomes by sub-group



*N=total number contributing per time period, PY=person years, IR=Incidence rate per 100 person-years, HR=hazard ratio, CI=confidence interval, PMC=post-discharge malaria chemoprevention.

Secondary outcomes

PMC was associated with a 37% (HR=0.63, 0.52-0.77, p<0.001) reduction in the number of all-cause hospital re-admissions by 26 weeks. The effect was only evident during the PMC period (HR=0.31, 0.22-0.43, p<0.001), not during the post-intervention period (HR=1.09, 0.83-1.42, p=0.54) (Figure 2). Hospital readmissions due to severe malaria, severe anaemia, severe malarial anaemia, and other severe diseases were also significantly reduced, overall, and during the PMC period. All-cause mortality was similar overall by 26 weeks (HR=0.74, 0.35-1.56, p=0.43). There was a 92% reduction in mortality during the intervention period (HR=0.08, 0.01-0.64, p=0.02), but this was higher (not significant) during the post-intervention period (HR=2.67, 0.85-8.40, p=0.09) (Figure 2).

Among the non-severe outcomes, PMC was associated with significantly lower risks of all-cause sick-child out-patient clinic visits mainly reflecting reductions in uncomplicated malaria (Figure 2) during the PMC period (HR=0.31, 0.23-0.41, p<0.001). There was no effect on non-malaria sick-child clinic visits. At the cross-sectional survey at 26 weeks, the groups did not differ in the prevalence of malaria infection, haemoglobin concentrations, or severe or moderate anaemia.

Adherence and tolerance outcomes

All 1,049 participants received the first course at randomisation, and 98.4% (1,031/1,048) and 96.8% (1,008/1,041) received the second and third PMC course at approximately six- and ten-weeks post-discharge. This was equally divided by the two arms (Table S4). The study medications were well tolerated; 153 (29.2%) of children in the PMC arm and 168 (32.0%) in the placebo arms experienced an adverse event (AE) on days 1-5 after drug intake. The proportion of participants who vomited the study medication at least once within 60 minutes during the three courses after drug intake was higher in the PMC (65/524, 12.4%) than the placebo (20/525, 3.8%) arms.

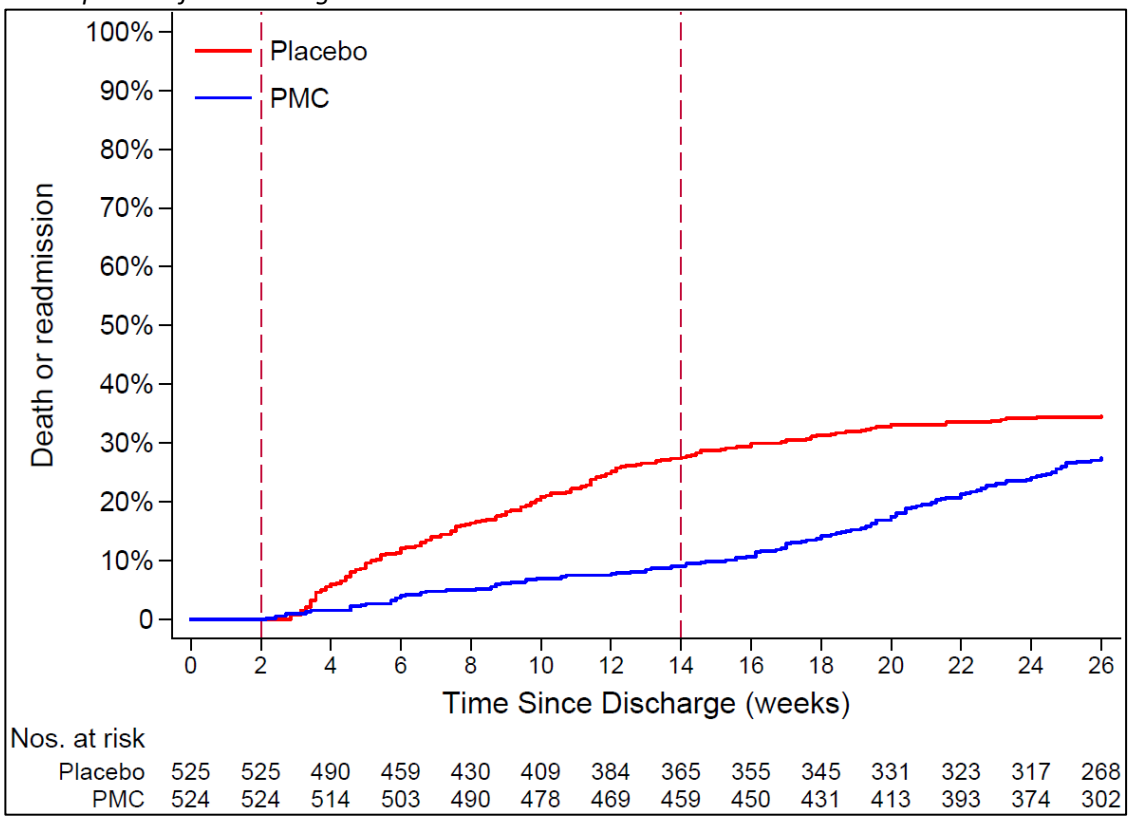
Safety outcomes

In the safety population severe adverse events (SAEs), other than the primary efficacy outcomes, occurred in 245 of 524 (46.8%) participants in the PMC arm compared with 319 of 525 (60.8%) participants in the placebo arm (Table S6). No severe adverse events were judged to be related to the trial drugs. Severe malaria was the most frequent adverse event reported in both arms. The incidence of adverse events did not significantly differ between the two groups.

Verbal autopsy and clinical notes suggested that none of the 28 deaths was attributed to the study medication; eight were due to severe malarial anaemia, nine were due to severe anaemia, and eleven were due to other causes.

ECG monitoring in 66 children showed that DP (N=33) was associated with an 18.6ms (95% CI 15.6-21.8) increase in the QTcF interval after the third dose of each course, whereas placebo-DP (N=33) was not (-1.8ms, -5.3-1.7) (p<0.001). The mean QTcF prolongation decreased with each subsequent course and was significantly lower after the third compared to the first courses of PMC with DP (p=0.02, Table S7). None of the 33 children in the DP arm experienced QTcF values >480ms. Sensitivity analysis using Bazett's method showed that 1 out of 33 children (3.0%) had an asymptomatic QTcB value >500ms (503ms) (Table S7).

Figure 4: Survival curve comparing time to the first event in PMC and Placebo arms over the 26 weeks period after discharge



Kaplan–Meier Estimates of time to event. Time (0 days) denotes the day of discharge. The dashed vertical lines denote the day of randomisation at 14 days post-discharge, and the end of the PMC intervention period at the end of week 14.

Discussion

About one-third of participants enrolled in the placebo arm died or were readmitted at least once during the 24 weeks after randomisation and many of them were readmitted multiple times confirming the high burden of post-discharge morbidity seen in previous studies in similar settings.[3,4,11,14,15] Most readmissions in the placebo arm were due to severe malaria or severe malarial anaemia and explain the impressive 35% protection against all-cause readmissions and deaths conferred by three months of malaria chemoprevention. The effect was restricted to the 12-week intervention period when the reduction was 70%, and not sustained after waning of the direct pharmacodynamic effects of the protective drug levels. Similar conclusions could be drawn from covariate-adjusted analyses, per-protocol analyses, and sensitivity analyses using alternative methods such as count or alternative time-to-event models.

We chose dihydroartemisinin-piperaquine for use in PMC because it is safe and well-tolerated by children and very effective as seasonal malarial chemoprevention or monthly intermittent preventive therapy in children.[16-19] The piperaquine component, which is eliminated slowly, provides at least four weeks of post-treatment prophylaxis allowing for the pragmatic 4-weekly or monthly administration used in this study. The previous PMC trial used three monthly courses of artemether-lumefantrine, which provided approximately three weeks of prophylaxis after each monthly course and 41% reduction in the same primary outcome during the 3-month chemoprevention period.[7] One other PMC trials in The Gambia, also conducted in recently hospitalized children with severe anaemia, showed that near-complete malaria prophylaxis provided post-discharge for the remainder of the malaria transmission season with weekly malaria prophylaxis with pyrimethamine-dapsone,[20] or monthly sulphadoxine-pyrimethamine[21] reduced all-cause readmission[20] or readmission due to severe anaemia[21] by 78%.

We continued follow-up for three months after the protective drug-levels had waned to determine the duration of any initial beneficial effect of PMC and whether this was cancelled out by a subsequent increased risk of events, e.g., through the loss or delayed acquisition of premunition. There was no evidence of either long-term protection or increased risk of uncomplicated clinical malaria following cessation of the intervention. However, there was an increase, albeit non-significant, in all-cause mortality (HR 2.67, p=0.09) consistent with previous seasonal malaria chemoprevention studies in children.[22,23] This could reflect an effect on

premunition but also an artifactual increase due to frailty effects because, by contrast to the placebo arm, the most vulnerable children in the PMC arm may have survived the intervention period. Overall, however, the protection conferred by PMC during the intervention period far outweighed any increased risk of events during the extended follow-up period. The effects of this intervention are restricted to malaria-associated morbidities, and there was no impact of non-malaria associated events, reflects that malaria was the predominant, but not the only cause of severe anaemia in this setting. Additional interventions such as antibiotics, micronutrient and multivitamins supplementations that target other aetiological factors may improve the efficacy of the intervention and address the complex and multifactorial nature of the aetiology of severe anaemia in this setting.

DP was well tolerated, and no serious adverse events attributable to the study drug were reported during the intervention period. The incidence of mild adverse events was also similar in the PMC and placebo groups, except for asymptomatic corrected QT interval prolongation, which, as expected, was significantly higher with dihydroartemisinin-piperaquine than placebo. However, no episode of QTc prolongation was associated with arrhythmias or clinical adverse events, consistent with other studies using monthly dihydroartemisinin-piperaquine in pregnant women[24] or other groups.[18] Only a few mothers/caretakers withdrew their children because of perceived adverse events associated with the intervention, and this was equally divided between both arms.

Nevertheless, like SMC,[22] when PMC is implemented, clear health education messages would need to be delivered to PMC providers and the target population to achieve effective coverage of PMC under programmatic conditions. Recent health services research show that PMC is likely to be highly acceptable by mothers/caretakers and that community delivery (i.e. all PMC doses are given to the mother/caretaker on discharge), ideally when combined with SMS reminders, is more likely to achieve high coverage than facility-based delivery that requires mothers/caretakers to return to the clinic for the 2nd and 3rd PMC course.[25,26] Modeling studies to determine the epidemiological and geographical settings where PMC would be a cost-effective intervention are merited.

Potential limitations include the relatively short post-intervention follow-up period. Another potential limitation is the use of a composite primary endpoint; the relative risk reduction, and

particularly the reduction in absolute risk differed considerably between the two components (i.e., death vs. non-fatal hospital admissions), which combined with the fact that they may be of different importance to policymakers and mothers/caretakers, makes the interpretation of the composite endpoint more challenging than the individual components. This study was not designed to assess the effect of iron supplementation on the potential risk of adverse events due to bacterial infections or malaria because both arms received iron as per national guidelines.

The results of this trial are very encouraging and suggest that in areas with intense malaria transmission, three months of malaria chemoprevention in children recently admitted with severe anaemia is a highly effective intervention resulting in substantial benefits in reducing all-cause readmissions or death post-discharge.

Article information

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Declaration of interests

There are no conflicts of interest to declare. The study drug dihydroartemisinin-piperaquine (Eurartesim®) and the placebo were kindly provided free of charge by the manufacturer Alfasigma (previously Sigma-Tau), Rome, Italy.

Data sharing statement

All individual-participant data collected during this trial will be available to access, after de-identification. Data and documents, including the study protocol, and the statistical analysis plan will be available. Data access will be provided to researchers after a proposal has been approved by an independent review committee identified for this purpose. An agreement on how to

collaborate will be reached based on any overlap between the proposal and any ongoing efforts. Data will be available beginning at three months after publication of this Article. Proposals should be directed to feiko.terkuile@lstmed.ac.uk and Bjarne.Robberstad@uib.no; to gain access, data requesters will need to sign a data access agreement, and the de-identified database will be transferred by email.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to:

Kwambai TK, Dhabangi A, Idro R, Opoka R, Kariuki S, Watson V, Ashitiba N, Otieno K, Samuels AM, Desai M, Boele van Hensbroek M, Wang D, Chandy J, Robberstad B, Phiri K, ter Kuile FO.

Monthly malaria chemoprevention for the post-discharge management of severe anaemia in children

Supplemental methods

Eligibility criteria

Children aged <5 years, weighing ≥5 kg, who had been admitted with severe anaemia (haemoglobin <5g/dL/haematocrit<15% or clinical indication for blood transfusion) and had received the standard in-hospital care were eligible during their convalescence period if their post-transfusion haemoglobin concentration was >5g/dL, they were clinically stable and able to take oral medication and if their caretakers agreed to the follow-up schedule. Exclusion criteria included severe anaemia due to blood loss/trauma, malignancy, known bleeding disorders or sickle cell disease. Children with these specific causes of severe anaemia were excluded because currently there are clear guidelines for their management in-hospital and during the follow-up periods, both in Uganda and Kenya. Other exclusion criteria included known hypersensitivity to study drug, known heart conditions or need for drugs that may be associated with QTc prolongation, non-resident in the study area, enrolled into another clinical trial or previous participation in this trial.

Outcomes

Efficacy outcomes

Primary efficacy outcome

The composite primary outcome of all-cause mortality or all-cause hospital readmissions determined by the time from randomisation around two weeks post-discharge until week-26 inclusive. A composite outcome was chosen to increase the power of the study and because the two components were expected to follow a similar biological pathway and have similar relative risk reductions.

Secondary efficacy outcomes

- The composite primary outcome of all-cause mortality or all-cause hospital readmissions by study period (PMC-intervention period and PMCpost-intervention period)
- All-cause hospital readmissions (overall and by study period)

- Hospital readmissions due to severe anaemia or malaria (composite) (overall and by study period)
- All-cause mortality (overall and by study period)
- All-cause sick clinic visits (overall and by study period)
- Uncomplicated clinical malaria (overall and by study period)

Tertiary efficacy outcomes

- Hospital readmissions due to severe anaemia (overall and by study period)
- Hospital readmissions due to severe malarial anaemia (overall and by study period)
- Hospital readmissions due to severe malaria (overall and by study period)
- Hospital readmission due to severe disease other than severe anaemia and severe malaria
- Non-severe all-cause sick-child clinic visits (overall and by study period)
- Non-malaria sick child clinic visits (overall and by study period)
- Malaria infection (overall and by study period)
- Malaria infection (at 26 weeks)
- Hb (at 26 weeks)
- Any anaemia (Hb<11 g/dL), mild anaemia (Hb 8.0-10.99 g/dL) moderate anaemia (Hb 5.0-7.99 g/dL) and severe anaemia (Hb<5 g/dL) (at 26 weeks)
- Weight-for-age, height-for-age, and height-for-weight z-scores (standard deviation [SD] scores of reference population) and as categories <2SD and <3SD from references population (at 26 weeks).

Tolerability and safety outcomes

- Mean QTc and mean increase in QTc prolongation measured by electrocardiogram (ECG) 4-6 hours after 3rd dose of each course (mean SD) and QTC prolongation >480ms and >500ms and >60ms increase in QTc at any course (binary; yes/no)

Statistical Analysis

Definition of study time intervals

Participants were randomised about 14 (11-28) days after discharge. This was delayed if the child was readmitted before day 14 or was found to be acutely ill on the scheduled day of randomisation. The survival or incidence data analysis cut-off date for a participant was; the date of last study visit if no follow-up contact was available thereafter or last known survival status date or end of study visit at 182 days (26 weeks) post-discharge (i.e. enrolment).

The study period was divided into three periods; 1) pre-randomisation screening period of approximately 2 weeks from the time the participant was formally enrolled (day 0) until the day of randomisation when the first dose of the first course of PMC or PMC-placebo was given, 2) PMC-intervention period (week 3-14) of approximately 12 weeks starting from the date of randomisation and ending 4 weeks (28 days inclusive) after the date of the first dose of the last 3-day course of PMC or 14 weeks from enrolment (98 days inclusive), whichever came last and 3) PMCpost-intervention period (weeks 15-26) of approximately 12 weeks from the end date of the subject PMC intervention period plus one day until 26 weeks after enrolment (182 days).

The analysis for each outcome was presented as the overall effect during the entire on-study period (3-26 weeks) and stratified by study period (the PMC intervention period and the PMC post-intervention period).

Covariate adjusted analysis

Secondary covariate-adjusted analysis of the primary outcome was conducted to determine whether the treatment effect estimates, or standard errors were affected by the inclusion of covariables. In addition to stratification factor, site and weight, the following pre-specified covariates were included: previous hospital admissions, the syndrome on admissions (non-malaria severe anaemia vs. severe malarial anaemia, age, Hb level at randomisation, distance to hospital, readmission during the pre-randomisation screening period, and socio-economic status. The same covariates were used in the sub-group analysis of the primary outcome defined *a priori*.

Subgroup analysis of the primary outcome

Subgroup analysis of the primary outcome using PWP-TT model was conducted to determine the extent to which the study effect varied by sub-groups. The treatment, subgroup variable, and their interaction term were used as predictors and the P-value for the interaction term obtained from the interaction term models (for those variables not involving period) or using Altman and Bland's method for models involving interaction with the period of assessment. Sub-group variables considered *a priori* included; period of assessment, adherence to the number of courses taken, malarial season on admission based on microscopy or RDT positivity among participants, country, dose in mg/kg received, the severity of anaemia on initial admission, blood transfusion status on initial admission, Hb level at randomisation, distance to nearest study hospital and socioeconomic status.

Cause of death

Data on cause of deaths was collected at least one month after the death had occurred using standardized WHO verbal autopsy forms,[1] to gather information on symptoms and any treatments or care sought prior to death. The forms were reviewed independently by two clinical officers and a probable cause of death assigned. The number (%) of participants with cause-specific death was then summarized by treatment group.

Results of cardiac monitoring

A total of 66 children were recruited; 33 per arm. All 66 received three courses of PMC. A total of 1,188 ECGs were taken consisting of 396 timepoints in triplicate, 2 per course for three courses each. There were no clinical cardiac adverse events.

The Fridericia's method resulted in a better rate correction than the Bazett's method in the PMC arm. In the PMC arm, the mean (SD) QTcF was 400ms (14) at baseline and increased to 422ms (15), 4-6 hours after the last dose of the first course (Table S7), representing a mean (SD) increase of 22ms (14) (95% CI 20 to 24) (range -6 to 54), $p<0.0001$ (paired t-test). No increase in QTc values was seen in the placebo arm.

Significant increases were also seen after the second and third course. Comparison of the delta QTc between the courses suggested a significant decline in QTcF prolongation from a mean (SD) of 22ms (14) after the first course to 14ms (17) after the third course (mean difference [MD],

8ms, 95% CI 1-15, p=0.02, by paired t-test). None of the children had QTcF values exceeding 480ms.

Supplemental references

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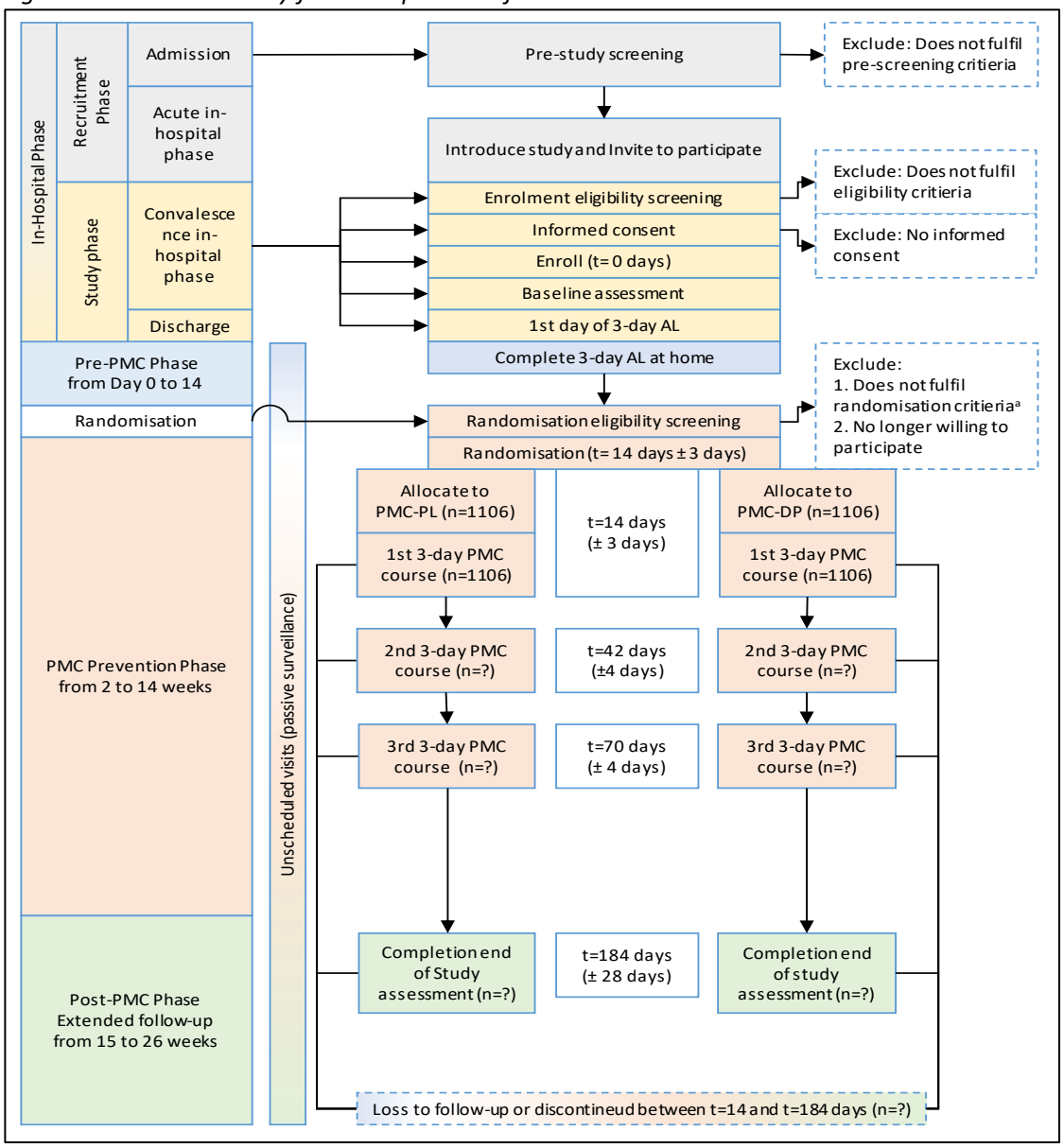
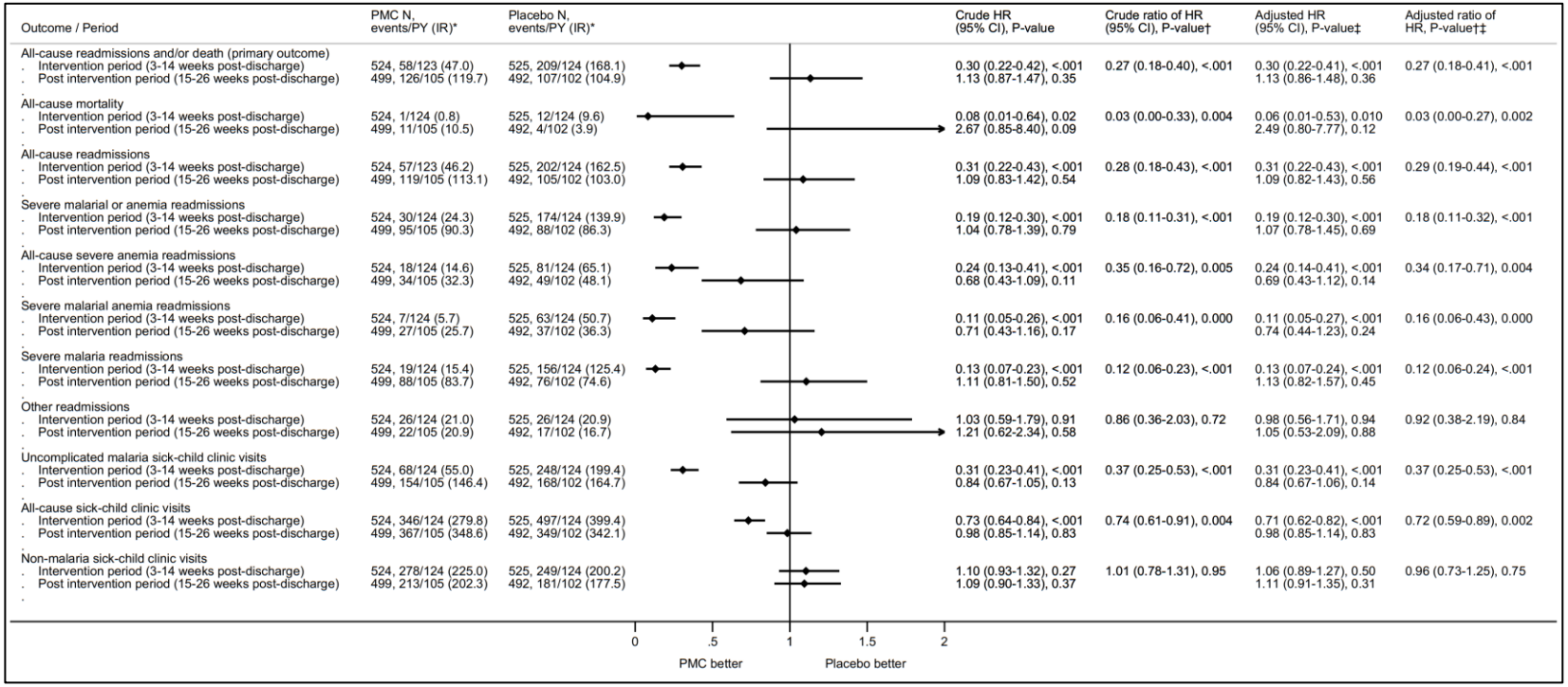
Supplemental figures

Figure S1: Overview study flow and periods of assessment



Children who fulfil the enrolment criteria but not the randomisation criteria will not be randomised, but where feasible will continued to be followed until the end of study at 26 weeks
PMC, post-discharge malaria chemoprevention; DP, dihydroartemisinin-piperaquine; PL, placebo.

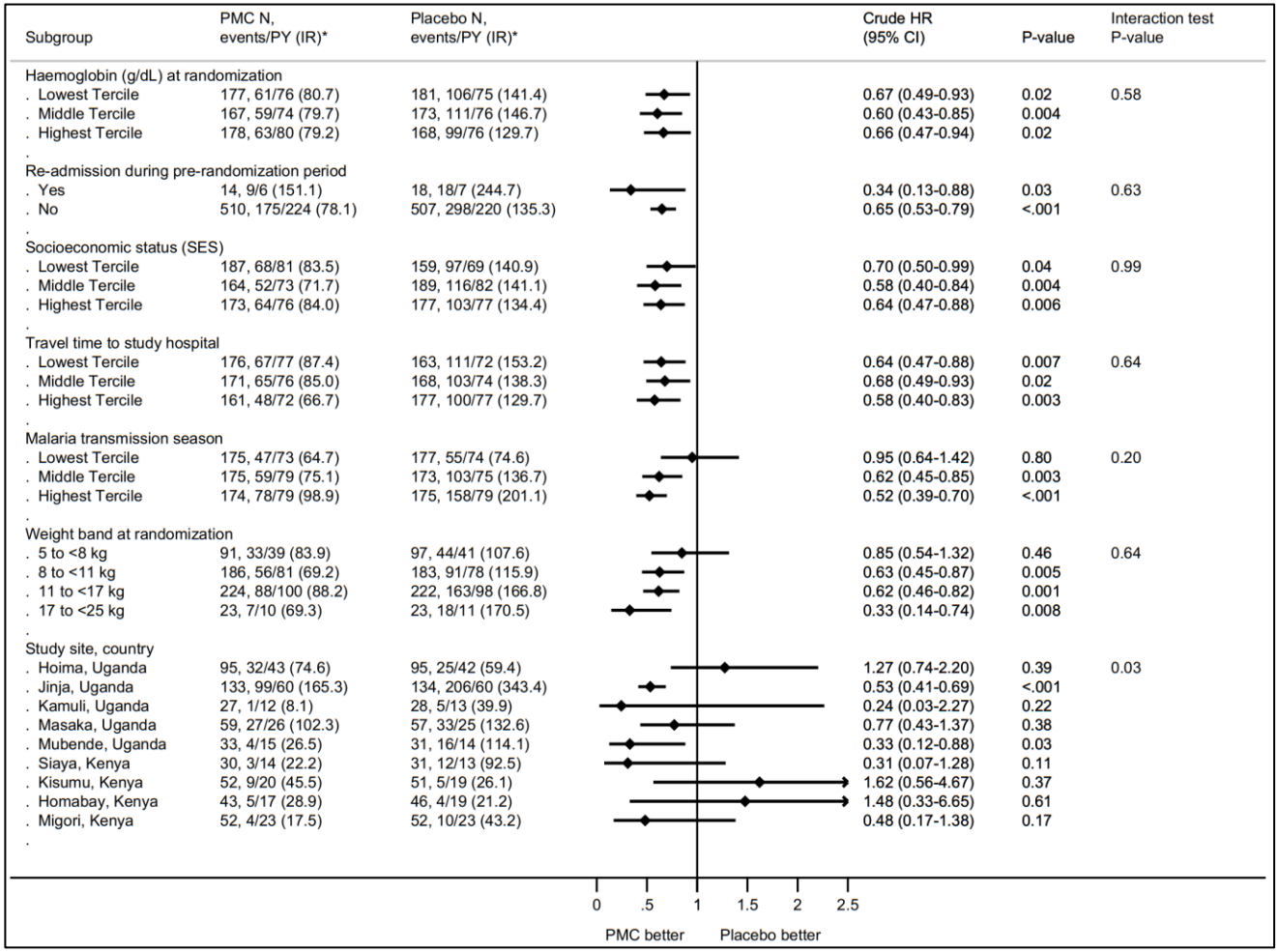
Figure-S2: interaction terms for period and treatment



N=total number contributing per period, PMC=post-discharge malaria chemoprevention, IR=incidence rate per 100 person-years, PY=person years, HR=hazard ratio, CI=confidence interval.

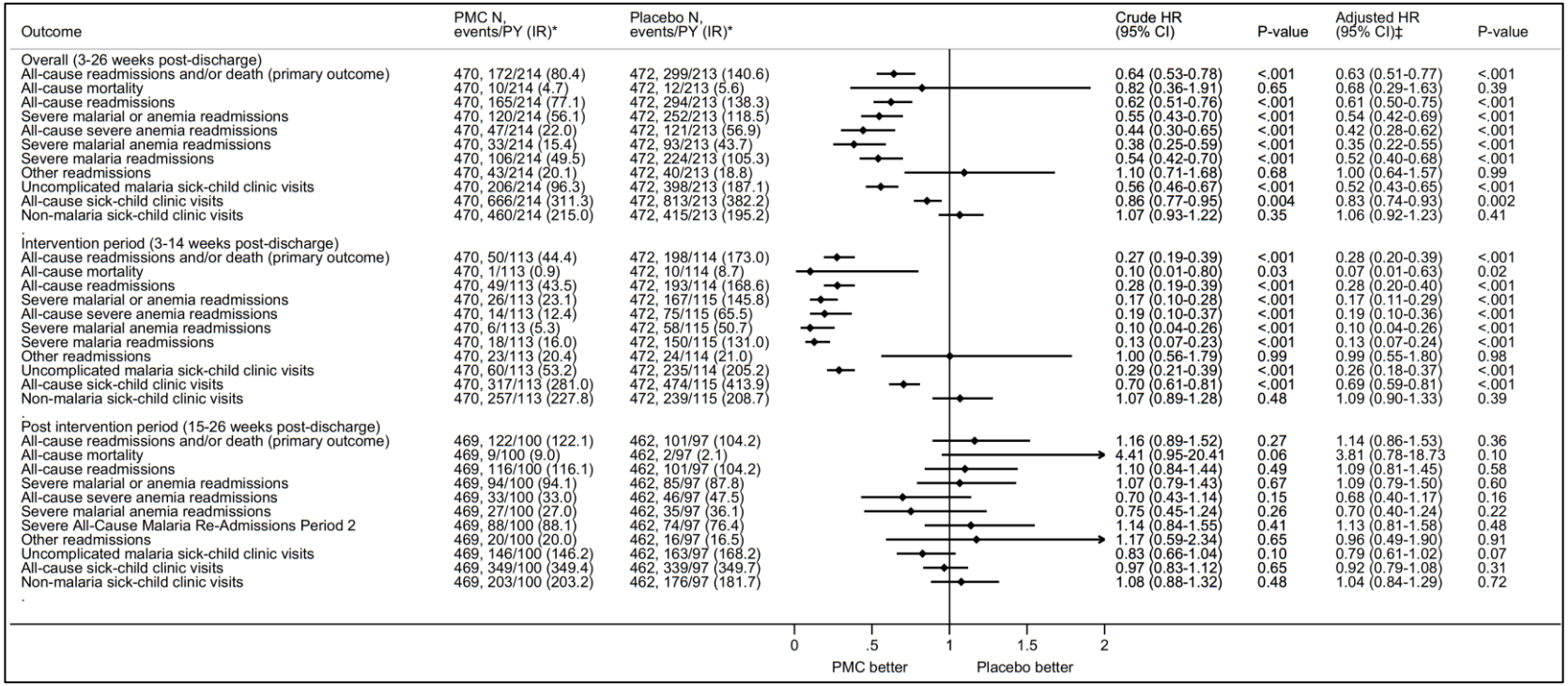
* Incidence rates obtained from two separate models; one per period. § Ratio of the hazard ratio in the intervention period and the post-intervention period obtained using the method described by Altman and Bland.[4] ‡ Adjusted for site, bodyweight, previous hospital admissions, the syndrome on admissions, age, haemoglobin level at randomization, distance to hospital, number of previous admissions, and socio-economic status.

Figure-S3: sub-group analysis by participant characteristics



PMC=post-discharge malaria chemoprevention; IR=incidence rate per 100 person-years; PY=person-years; HR=hazard ratio; CI=confidence interval.

Figure-S4: Per protocol analysis



Outcome	PMC N, events/PY (IR)*	Placebo N, events/PY (IR)*	Crude HR (95% CI)	P-value	Adjusted HR (95% CI)‡	P-value
Overall (3-26 weeks post-discharge)						
All-cause readmissions and/or death (primary outcome)	470, 172/214 (80.4)	472, 299/213 (140.6)	0.64 (0.53-0.78)	<.001	0.63 (0.51-0.77)	<.001
All-cause mortality	470, 10/214 (4.7)	472, 12/213 (5.6)	0.82 (0.36-1.91)	0.65	0.68 (0.29-1.63)	0.39
All-cause readmissions	470, 165/214 (77.1)	472, 294/213 (138.3)	0.62 (0.51-0.76)	<.001	0.61 (0.50-0.75)	<.001
Severe malarial or anemia readmissions	470, 120/214 (56.1)	472, 252/213 (118.5)	0.55 (0.43-0.70)	<.001	0.54 (0.42-0.69)	<.001
All-cause severe anemia readmissions	470, 47/214 (22.0)	472, 121/213 (56.9)	0.44 (0.30-0.65)	<.001	0.42 (0.28-0.62)	<.001
Severe malarial anemia readmissions	470, 33/214 (15.4)	472, 93/213 (43.7)	0.38 (0.25-0.59)	<.001	0.35 (0.22-0.55)	<.001
Severe malaria readmissions	470, 106/214 (49.5)	472, 224/213 (105.3)	0.54 (0.42-0.70)	<.001	0.52 (0.40-0.68)	<.001
Other readmissions	470, 43/214 (20.1)	472, 40/213 (18.8)	1.10 (0.71-1.68)	0.68	1.00 (0.64-1.57)	0.99
Uncomplicated malaria sick-child clinic visits	470, 206/214 (96.3)	472, 398/213 (187.1)	0.56 (0.46-0.67)	<.001	0.52 (0.43-0.65)	<.001
All-cause sick-child clinic visits	470, 666/214 (311.3)	472, 813/213 (382.2)	0.86 (0.77-0.95)	0.004	0.83 (0.74-0.93)	0.002
Non-malaria sick-child clinic visits	470, 460/214 (215.0)	472, 415/213 (195.2)	1.07 (0.93-1.22)	0.35	1.06 (0.92-1.23)	0.41
Intervention period (3-14 weeks post-discharge)						
All-cause readmissions and/or death (primary outcome)	470, 50/113 (44.4)	472, 198/114 (173.0)	0.27 (0.19-0.39)	<.001	0.28 (0.20-0.39)	<.001
All-cause mortality	470, 1/113 (0.9)	472, 10/114 (8.7)	0.10 (0.01-0.80)	0.03	0.07 (0.01-0.63)	0.02
All-cause readmissions	470, 49/113 (43.5)	472, 193/114 (168.6)	0.28 (0.19-0.39)	<.001	0.28 (0.20-0.40)	<.001
Severe malarial or anemia readmissions	470, 26/113 (23.1)	472, 167/115 (145.8)	0.17 (0.10-0.28)	<.001	0.17 (0.11-0.29)	<.001
All-cause severe anemia readmissions	470, 14/113 (12.4)	472, 75/115 (65.5)	0.19 (0.10-0.37)	<.001	0.19 (0.10-0.36)	<.001
Severe malarial anemia readmissions	470, 6/113 (5.3)	472, 58/115 (50.7)	0.10 (0.04-0.26)	<.001	0.10 (0.04-0.26)	<.001
Severe malaria readmissions	470, 18/113 (16.0)	472, 150/115 (131.0)	0.13 (0.07-0.23)	<.001	0.13 (0.07-0.24)	<.001
Other readmissions	470, 23/113 (20.4)	472, 24/114 (21.0)	1.00 (0.56-1.79)	0.99	0.99 (0.55-1.80)	0.98
Uncomplicated malaria sick-child clinic visits	470, 60/113 (53.2)	472, 235/114 (205.2)	0.29 (0.21-0.39)	<.001	0.26 (0.18-0.37)	<.001
All-cause sick-child clinic visits	470, 317/113 (281.0)	472, 474/115 (413.9)	0.70 (0.61-0.81)	<.001	0.69 (0.59-0.81)	<.001
Non-malaria sick-child clinic visits	470, 257/113 (227.8)	472, 239/115 (208.7)	1.07 (0.89-1.28)	0.48	1.09 (0.90-1.33)	0.39
Post intervention period (15-26 weeks post-discharge)						
All-cause readmissions and/or death (primary outcome)	469, 122/100 (122.1)	462, 101/97 (104.2)	1.16 (0.89-1.52)	0.27	1.14 (0.86-1.53)	0.36
All-cause mortality	469, 9/100 (9.0)	462, 2/97 (2.1)	4.41 (0.95-20.41	0.06	3.81 (0.78-18.73	0.10
All-cause readmissions	469, 116/100 (116.1)	462, 101/97 (104.2)	1.10 (0.84-1.44)	0.49	1.09 (0.81-1.45)	0.58
Severe malarial or anemia readmissions	469, 94/100 (94.1)	462, 85/97 (87.8)	1.07 (0.79-1.43)	0.67	1.09 (0.79-1.50)	0.60
All-cause severe anemia readmissions	469, 33/100 (33.0)	462, 46/97 (47.5)	0.70 (0.43-1.14)	0.15	0.68 (0.40-1.17)	0.16
Severe malarial anemia readmissions	469, 27/100 (27.0)	462, 35/97 (36.1)	0.75 (0.45-1.24)	0.26	0.70 (0.40-1.24)	0.22
Severe All-Cause Malaria Re-Admissions Period 2	469, 88/100 (88.1)	462, 74/97 (76.4)	1.14 (0.84-1.55)	0.41	1.13 (0.81-1.58)	0.48
Other readmissions	469, 20/100 (20.0)	462, 16/97 (16.5)	1.17 (0.59-2.34)	0.65	0.96 (0.49-1.90)	0.91
Uncomplicated malaria sick-child clinic visits	469, 146/100 (146.2)	462, 163/97 (168.2)	0.83 (0.66-1.04)	0.10	0.79 (0.61-1.02)	0.07
All-cause sick-child clinic visits	469, 349/100 (349.4)	462, 339/97 (349.7)	0.97 (0.83-1.12)	0.65	0.92 (0.79-1.08)	0.31
Non-malaria sick-child clinic visits	469, 203/100 (203.2)	462, 176/97 (181.7)	1.08 (0.88-1.32)	0.48	1.04 (0.84-1.29)	0.72

* N=total number contributing per period, PY=person years, IR=incidence rate per 100 person-years, HR=hazard ratio

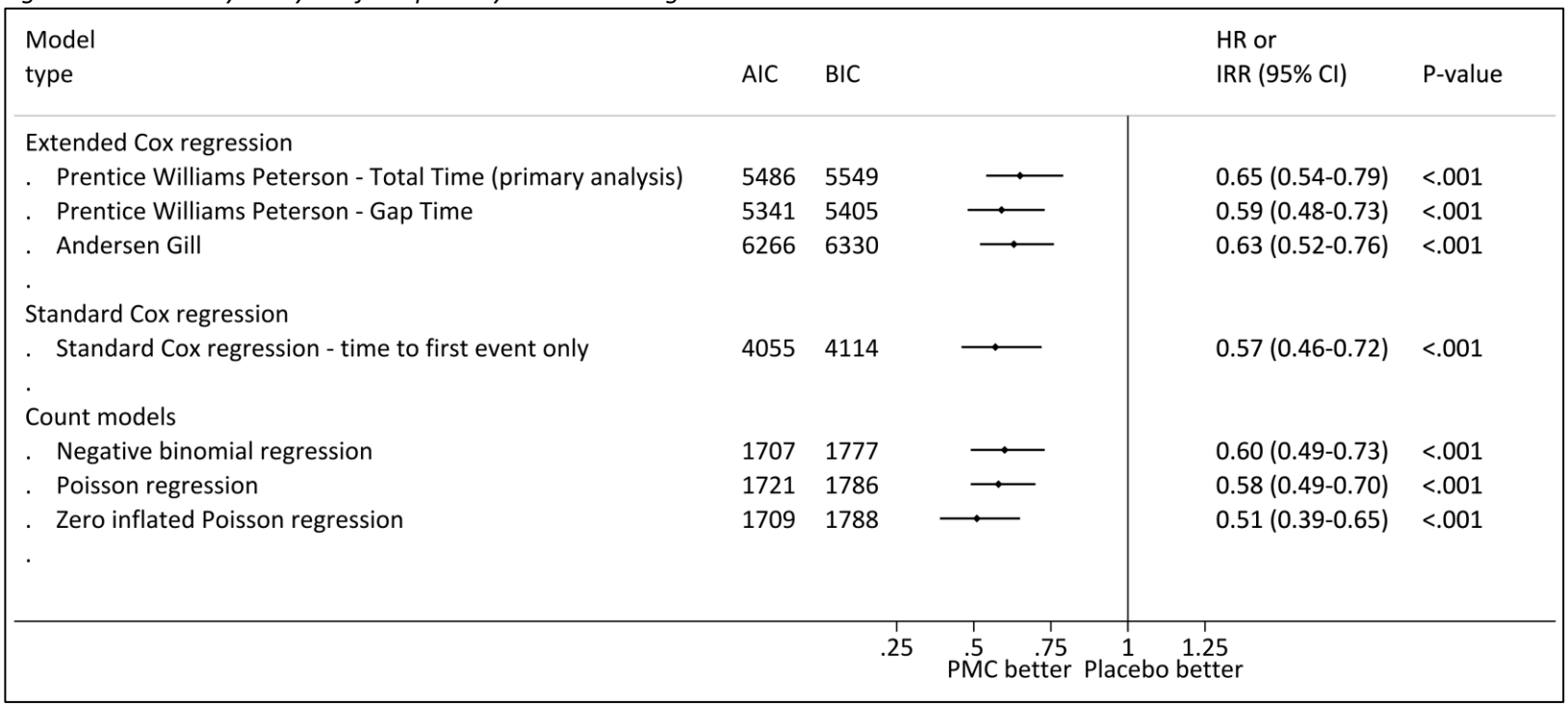
† Median and interquartile range (IQR) of the time to any event (i.e., first, second, third event, etc. pooled).

‡ Adjusted for site, bodyweight, previous hospital admissions, the syndrome on admissions, age, haemoglobin level at randomization, distance to hospital, number of previous admissions, and socio-economic status.

PMC=Post-discharge malaria chemoprevention

Confidence intervals for secondary outcomes have not been adjusted for multiple testing, and inferences drawn from the intervals may not be reproducible.

Figure-S5: Sensitivity analysis of the primary outcome using alternative models



HR=hazard rate, IRR=incidence rate ratio, AIC=Akaike information criterion, BIC=Bayesian information criterion.

Supplemental tables

Interventions

Table S1: Artemether-lumefantrine weight-based dosing schedule

Weight in Kg	Number of pediatric tablets of artemether (20 mg)-and lumefantrine (120 mg) per dose					
	Day 1 1st dose	8 hours	Day 2 24 hours	36 hours	Day 3 48 hours	60 hours
5 to <15	1	1	1	1	1	1
15 to<25	2	2	2	2	2	2
25 to ≤34	3	3	3	3	3	3
>34	4	4	4	4	4	4

Table S2: Dihydroartemisinin-piperaquine weight-based dosing schedule

Weight in Kg	Daily dose (mg)		Tablet strength and number of tablets per dose
	Piperaquine	DHA	
5 to <8	160	20	1 x 160 mg / 20 mg tablet or ½ x 320 mg / 40 mg tablet
8 to <11	240	30	1.5 x 160 mg / 20 mg tablet or ¾ x 320 mg / 40 mg tablet
11 to <17	320	40	1 x 320 mg / 40 mg tablet
17 to <25	480	60	1.5 x 320 mg / 40 mg tablet
25 to<36	640	80	2 x 320 mg / 40 mg tablet

DHA, dihydroartemisinin;

*Table S3: Full list of baseline characteristics of study participants by country. **

	Uganda				Kenya			
Child characteristics	PMC (N=347)		Placebo (N=345)		PMC (N=177)		Placebo (N=180)	
Sex (male)—no./total no. (%)	181/347	(52.2)	168/345	(48.7)	99/177	(55.9)	97/180	(53.9)
Age—Mean (SD)	27.4±14.8		27.3±14.5		25.7±16.7		24.5±15.3	
Distance (KM)—Mean (SD)	31.4±23.9		31.4±23.4		19.2±17.0		24.2±23.8	
SES—no./total no. (%)								
poor	95/332	(28.6)	72/325	(22.2)	30/124	(24.2)	25/125	(20.0)
poorer	147/332	(44.3)	172/325	(52.9)	65/124	(52.4)	73/125	(58.4)
poorest	90/332	(27.1)	81/325	(24.9)	29/124	(23.4)	27/125	(21.6)
Hb Level (g/dL) pre-transfusion- Mean (SD)	4.2±1.0		4.1±1.2		4.1±1.2		4.2±1.2	
Hb level (g/dL) at enrolment-Mean (SD)	7.7±1.7		8.0±1.8		8.9±7.3		8.0±2.4	
Weight (Kg) at randomization- Mean (SD)	11.0±3.0		10.9±3.0		11.3±3.5		11.1±3.4	
Malaria RDT at enrolment—no./total no. (%)								
Positive	250/347	(72.0)	238/345	(69.0)	7/177	(4.0)	10/180	(5.6)
Negative	40/347	(11.5)	36/345	(10.4)	5/177	(2.8)	5/180	(2.8)
Not Done	57/347	(16.4)	71/345	(20.6)	165/177	(93.2)	165/180	(91.7)
HIV status—no./total no. (%)								
Positive	2/347	(0.6)	1/345	(0.3)	3/177	(1.7)	0/180	(0.0)
Negative	104/347	(30.0)	107/345	(31.0)	142/177	(80.2)	134/180	(74.4)
Not known	239/347	(68.9)	234/345	(67.8)	27/177	(15.3)	39/180	(21.7)
Exposed	2/347	(0.6)	3/345	(0.9)	5/177	(2.8)	7/180	(3.9)
Bednet use—no./total no. (%)	206/251	(82.1)	199/250	(79.6)	111/129	(86.0)	119/135	(88.1)
SMA—no./total no. (%)	303/347	(87.3)	307/345	(89.0)	133/177	(75.1)	147/180	(81.7)
Non-SMA—no./total no. (%)	37/347	(10.7)	36/345	(10.4)	37/177	(20.9)	25/180	(13.9)
Previous hospitalizations—no./total no. (%)								
1	206/347	(59.4)	203/344	(59.0)	133/174	(76.4)	127/178	(71.3)
2	69/347	(19.9)	65/344	(18.9)	25/174	(14.4)	33/178	(18.5)

>=3	72/347	(20.7)	76/344	(22.1)	16/174	(9.2)	18/178	(10.1)
Treated with antibiotics—no./total no. (%)	346/347	(99.7)	343/345	(99.4)	174/177	(98.3)	176/180	(97.8)
Difficulty in breathing—no./total no. (%)	133/347	(38.3)	137/345	(39.7)	31/177	(17.5)	24/180	(13.3)
History of fever—no./total no. (%)	334/347	(96.3)	338/345	(98.0)	170/177	(96.0)	173/180	(96.1)
HAZ—Mean (SD)-Mean (SD)	-1.0±3.9		-1.3±3.1		-0.1±4.9		-0.4±5.2	
WAZ—Mean (SD)	-0.7±2.0		-0.9±2.0		-0.3±2.1		-0.6±1.3	
WHZ—Mean (SD)	-0.2±1.2		-0.2±1.3		-0.1±1.7		-0.1±2.0	
HAZ <-2 z score	88/233	(37.8)	104/221	(47.1)	36/123	(29.3)	39/128	(30.5)
HAZ <-3 z score	31/233	(13.3)	43/221	(19.5)	19/123	(15.4)	18/128	(14.1)
WAZ <-2 z score	33/233	(14.2)	44/221	(19.9)	16/123	(13.0)	15/128	(11.7)
WAZ <-3 z score	8/233	(3.4)	17/221	(7.7)	7/123	(5.7)	5/128	(3.9)
WHZ <-2 z score	12/231	(5.2)	16/220	(7.3)	13/120	(10.8)	14/122	(11.5)
WHZ <-3 z score	3/231	(1.3)	5/220	(2.3)	7/120	(5.8)	4/122	(3.3)
Parental characteristics								
Marital Status—no./total no. (%)								
Single	11/347	(3.2)	15/345	(4.3)	30/177	(16.9)	14/180	(7.8)
Married	295/347	(85.0)	290/345	(84.1)	130/177	(73.4)	145/180	(80.6)
Other	41/347	(11.8)	40/345	(11.6)	17/177	(9.6)	21/180	(11.7)
Father employment status—no./total no. (%)	26/109	(23.9)	15/94	(16.0)	6/25	(24.0)	7/23	(30.4)
Mother education level—no./total no. (%)								
None	28/209	(13.4)	25/226	(11.1)	8/140	(5.7)	8/140	(5.7)
Completed primary	125/209	(59.8)	143/226	(63.3)	99/140	(70.7)	105/140	(75.0)
Completed secondary	53/209	(25.4)	56/226	(24.8)	31/140	(22.1)	16/140	(11.4)
Tertiary/University	3/209	(1.4)	2/226	(0.9)	2/140	(1.4)	11/140	(7.9)

SD, standard deviation; SES, socioeconomic status; RDT, rapid diagnostic test; HIV, human immune deficiency virus; SMA, severe malarial anaemia; HAZ, height for age z-score; WAZ, weight for age z-score; WHZ, weight for age z-score. There were no significant differences between the study groups in any characteristic. Percentages may not total to 100 because of rounding.

Table S4: Adherence table

	Uganda		Kenya		Overall		
	PMC N=347	Placebo N=345	PMC N=177	Placebo N=180	PMC N=524	Placebo N=525	Pooled N=1049
Achieved¶/expected€ (%)							
PMC1	347/347 (100.0)	345/345 (100.0)	177/177 (100.0)	180/180 (100.0)	524/524 (100.0)	525/525 (100.0)	1049/1049 (100.0)
PMC2	345/347 (99.4)	342/344 (99.4)	171/177 (96.6)	173/180 (96.1)	516/524 (98.5)	515/524 (98.3)	1031/1048 (98.4)
PMC3	345/347 (99.4)	336/341 (98.5)	164/176 (93.2)	163/177 (92.1)	509/523 (97.3)	499/518 (96.3)	1008/1041 (96.8)
Total person-days							
Overall (week 3-26)	56,042	54,226	26,830	26,740	82,872	80,966	163,838
PMC intervention period (week 3-14)	29,245	28,665	15,077	15,227	44,322	43,892	88,214
Post intervention period (week 15-26)	26,589	25,318	11,505	11,406	38,094	36,724	74,818
Median (IQR) person-days							
Overall (week 3-26)	168 (163-168)	166 (159-168)	168 (163-168)	168 (159-168)	168 (163-168)	168 (159-168)	168 (161-168)
PMC intervention period (week 3-14)	84 (84-86)	84 (81-86)	85 (84-93)	85 (84-95)	84 (84-87)	84 (82-88)	84 (83-88)
Post intervention period (week 15-26)	82 (76-83)	81 (74-83)	80 (69-83)	78 (64-83)	81 (75-83)	80 (71-83)	81(73-83)
Lost/withdrawn by 26 weeks (%)							
Overall (week 3-26)	7	4	37	32	44 (8.4)	36 (6.9)	80 (7.6)
PMC intervention period (week 3-14)	3	4	21	17	24 (4.6)	21 (4.0)	45 (4.3)
Post-intervention period (week 15-26)	4	0	16	15	20 (3.8)	15 (2.9)	35 (3.3)

PMC=post-discharge malaria chemoprevention, IQR interquartile range.

PMC1, PMC2 AND PMC3 are the first, second and third PMC courses respectively provided at approximately 2, 6, and 10 weeks post-discharge.

¶Number of participants who received the PMC courses

€Number expected to receive the PMC course excluding the death

*Table-S5: End of study cross-sectional survey**

Characteristic	PMC (N=473)	Placebo (N=472)	RR or MD (95% CI)*	p-value
Fever documented or in last 48h	67/470 (14.3)	80/471 (17.0)	0.84 (0.62-1.13)	0.25
Malaria RDT positivity	116/461 (25.2)	137/464 (29.5)	0.85 (0.69-1.05)	0.14
Anemia (Hb <11.0 g/dL)	197/458 (43.0)	188/463 (40.6)	1.06 (0.91-1.23)	0.46
Moderate anemia (Hb≥5.0 and <8.0 g/dL)	48/458 (10.5)	39/463 (8.4)	1.24 (0.83-1.86)	0.29
Hemoglobin in g/dL, mean (SD)	11.0 (2.1)	11.0 (2.2)	-0.03 (-0.31-0.24)	0.83
HAZ, mean (SD)	-1.0 (3.0)	-1.0 (3.2)	0.07 (-0.34-0.48)	0.74
WAZ, mean (SD)	-0.6 (1.6)	-0.7 (1.4)	0.05 (-0.14-0.25)	0.61
WHZ, mean (SD)	-0.1 (1.3)	0.0 (1.4)	-0.09 (-0.27-0.09)	0.35
HAZ, Z-score <-2	146/448 (32.6)	157/452 (34.7)	0.94 (0.78-1.13)	0.50
HAZ, Z-score <-3	60/448 (13.4)	73/452 (16.2)	0.83 (0.60-1.14)	0.25
WAZ, Z-score <-2	64/448 (14.3)	59/452 (13.1)	1.09 (0.79-1.52)	0.59
WAZ, Z-score <-3	18/448 (4.0)	12/452 (2.7)	1.51 (0.74-3.10)	0.26
WHZ, Z-score <-2	28/446 (6.3)	20/446 (4.5)	1.40 (0.80-2.45)	0.24
WHZ, Z-score <-3	8/446 (1.8)	6/446 (1.3)	1.33 (0.47-3.81)	0.59

Numbers denote n/N (%) unless otherwise indicated.
SD standard deviations, SES socioeconomic status, RDT rapid diagnostic test, Hb haemoglobin, HAZ height (or length) for age z score, WAZ weight for age z score, WHZ weight for height (or length) age z score, CI confidence interval.
* RR=relative risk for binary variables, MD=mean difference for continuous variables.

Table S6: Serious adverse events

	PMC			PMC-Placebo		
MedDRA System organ class	No with event	Total events	Incidence per 100 person-days (95% CI)	No with event	Total events	Incidence per 100 person-days (95% CI)
Overall	245	319	0.29 (0.26-0.32)	354	581	0.40 (0.39-0.46)
Blood and lymphatic system disorders	58	74	24.62 (20.20-30.01)	99	142	37.50 (32.91-42.68)
Cardiac disorders	1	1	0.33 (0.05-2.35)	0	0	
Ear and labyrinth disorders	1	1	0.33 (0.05-2.35)	1	1	0.30 (0.04-1.87)
Gastrointestinal disorders	3	3	1.00 (0.32-3.08)	5	5	1.30 (0.55-3.15)
General disorders and administration site conditions	13	13	4.33 (2.54-7.36)	15	15	4.00 (2.41-6.50)
Hepatobiliary disorders	1	1	0.33 (0.05-2.35)	1	1	0.30 (0.04-1.87)
Immune system disorders	0	0		1	1	0.30 (0.04-1.87)
Infections and infestations	124	180	59.89 (54.59-65.69)	172	347	91.60 (88.83-94.42)
Metabolism and nutrition disorders	9	9	2.99 (1.57-5.70)	4	4	1.10 (0.40-2.80)
Nervous system disorders	2	2	0.67 (0.17-2.65)	3	3	0.80 (0.26-2.44)
Renal and urinary disorders	7	7	2.33 (1.12-4.84)	17	24	6.30 (4.30-9.33)
Respiratory, thoracic and mediastinal disorders	24	26	8.65 (5.99-12.49)	36	38	10.00 (7.42-13.56)
Skin and subcutaneous tissue disorders	1	1	0.33 (0.05-2.35)	0	0	
Musculoskeletal and connective tissue disorders	1	1	0.33 (0.05-2.35)	0	0	

PMC, post-discharge malaria chemoprevention; CI, confidence interval, MedDRA, Medical Dictionary for Regulatory Activities.

Table S7: Cardiac monitoring; mean and mean change in QTcF at baseline and 4 to 6 hours after the third dose of each course

PMC											
			QTcF in ms				Change in QTcF from baseline* in ms				
PMC course	Day + time ECG	No	Mean (SD)	Range	>480ms n (%)	>500ms n (%)	Mean (SD)	Range	95% CI	p	>60 ms
1st	0	33	400 (14)	377-431	0 (0)	0 (0)	Ref				
1st	2+4h	33	422 (15)	396-456	0 (0)	0 (0)	22 (14)	-6, 54	17, 27	<0.001	0 (0)
2nd	0	33	401 (16)	363-431	0 (0)	0 (0)	Ref				
2nd	2+4h	33	420 (17)	388-460	0 (0)	0 (0)	20 (15)	-18, 44	14, 25	<0.001	0 (0)
3rd	0	33	402 (15)	369-431	0 (0)	0 (0)	Ref				
3rd	2+4h	33	416 (20)	384-458	0 (0)	0 (0)	14 (16)	-14, 55	8, 20	<0.001	0 (0)
Placebo											
			QTcF in ms				Δ in QTcF from baseline* in ms				
PMC course	Day + time ECG	No	Mean (SD)	Range	>480ms n (%)	>500ms n (%)	Mean (SD)	Min-max	95% CI	p	>60 ms
1st	0	33	401 (14)	370-439	0 (0)	0 (0)	Ref				
1st	2+4h	33	401 (14)	376-431	0 (0)	0 (0)	0 (13)	-27, 31	-4, 5	0.87	0 (0)
2nd	0	33	400 (13)	373-420	0 (0)	0 (0)	Ref				
2nd	2+4h	33	395 (16)	367-420	0 (0)	0 (0)	-4 (17)	-46, 27	-10, 2	0.18	0 (0)
3rd	0	33	400 (19)	358-433	0 (0)	0 (0)	Ref				
3rd	2+4h	33	398 (20)	320-425	0 (0)	0 (0)	-2 (22)	-82, 47	-9, 6	0.68	0 (0)

DP, dihydroartemisinin-piperaquine; ECG, electrocardiogram; QTc, QT interval on ECG corrected using the Fridericia method (QTcF); ms, milliseconds; SD, standard deviation; 2+4h, Day 2 plus 4 to 6 hours after the third (last) dose of each course of DP (Tmax),
; CI, confidence interval.
* Change in QTcF from baseline in ms. Baseline was the QTcF interval taken just before the first dose of each course of PMC with DP

Chapter 7: Discussions

Main findings

This thesis aimed to provide evidence of the burden of post-discharge mortality and morbidity among children less than five years of age who are admitted with all-cause severe anaemia and living in malaria-endemic areas of Africa. Following the impressive results from the previous trial in Malawi where artemether-lumefantrine was shown to provide substantial protection against morbidity and mortality post-discharge,[1] we aimed to provide confirmatory evidence with a longer-acting antimalarial to show that post-discharge malaria chemoprevention is an effective tool for the management of severe anaemia post-discharge in this setting.

By analysing historical data collected between 2008 and 2013 within the KEMRI/CDC HDSS located in an area with intense year-round malaria transmission in western Kenya (chapter 3), we found that children admitted with severe anaemia have a 2-fold higher risk of death within the first six months post-discharge than children admitted with other syndromes, except for severe acute malnutrition (3-fold). Furthermore, it is well documented that severe anaemia is a leading cause of mortality and carries substantial in-hospital mortality risk ranging from 4 to 12% in different epidemiological settings.[2,3] We found that children admitted with severe anaemia were more likely to die in the first six months after discharge than during their in-hospital stay. Similar findings were observed for children admitted with severe acute malnutrition. Severe anaemia and severe acute malnutrition commonly co-exist in this setting, and this was found to worsen the post-discharge mortality outcomes. These findings were further confirmed when we pooled the findings from similar studies conducted in comparable epidemiological settings in a systematic review and meta-analysis (Chapter 4). Thus, the unrecognised risk of post-discharge mortality in children with severe anaemia likely contributes substantially to the overall burden of child mortality in malaria-endemic areas of Africa.

The previous trial in Malawi provided the initial evidence that malaria chemoprevention is a promising intervention.[1] This showed that a substantial reduction in all-cause deaths or readmission among children with severe malaria anaemia could be achieved with three months of post-discharge malaria chemoprevention with artemether-lumefantrine.[1] In our current trial in Uganda and Kenya, which enrolled children with all-cause severe anaemia and which was designed to confirm the earlier finding from Malawi, three months of post-discharge chemoprevention with dihydroartemisinin-piperaquine (DP) was found to be highly efficacious (Chapter 6). PMC,

therefore, is a promising tool for the post-discharge management of children admitted with severe anaemia in malaria-endemic areas of Africa.

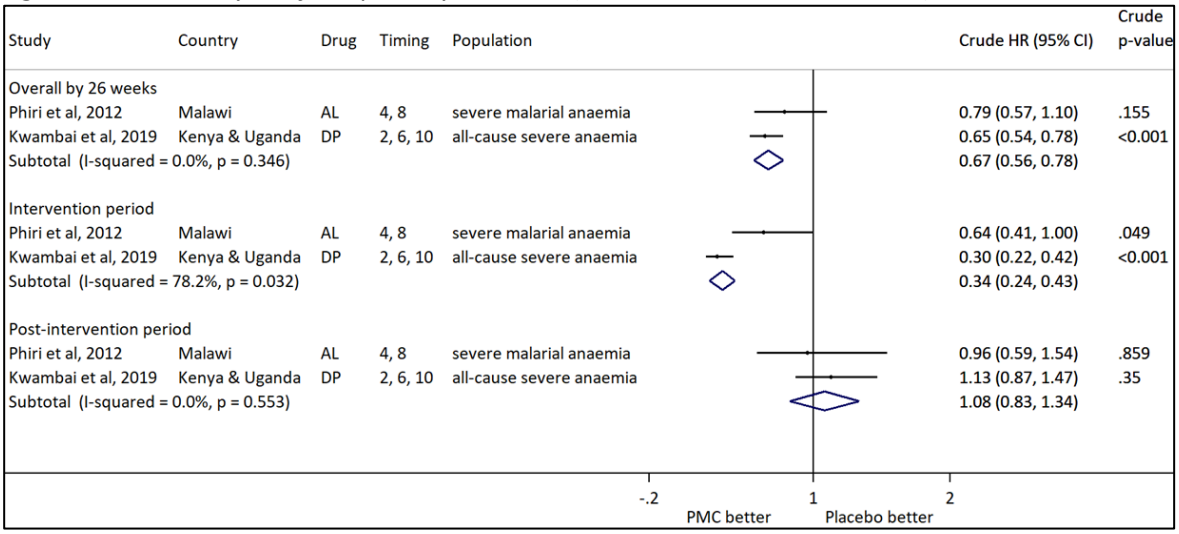
Efficacy of post-discharge malaria chemoprevention with dihydroartemisinin-piperaquine

Three monthly courses of DP administered to children living in a malaria-endemic area and recently discharged with severe anaemia was associated with a 35% reduction in all-cause death or all-cause readmission by six months. This was 70% during the three months intervention period. The effect was not sustained after the protective drug levels had waned (Chapter 6). The greatest reduction was seen in malaria-related re-admissions (i.e. severe malaria or severe malarial anaemia). With the multifactorial nature of the aetiology of severe anaemia in malaria-endemic areas, the results may vary in different epidemiological settings depending on the extent to which malaria contributes to the overall burden of severe anaemia visa vis other aetiological factors. These findings are better than those from the previous trial in Malawi which used artemether-lumefantrine and showed a 36% reduction for the same primary outcome during the three months intervention period, and 21% overall by 6 months (31% in multi-variate analysis).[1] We used DP which provided complete prophylaxis between the 4-weekly doses because of the longer post-discharge chemoprophylactic effects of the piperaquine component as opposed to the lumefantrine component which provides about three weeks of post-treatment prophylaxis. As opposed to the 69% reduction found in our trial, monthly courses with AL, provided more modest protection against clinical malaria (50%) during the intervention period in the Malawi trial. This may be related to the shorter duration of post-treatment prophylaxis that is achieved with the lumefantrine component which allows infections to occur in the last week before the next monthly course of the intervention is due. Our observed reduction in clinical malaria is consistent with the findings from earlier SMC trials which reported a 71% reduction with the SP and amodiaquine combination in Burkina Faso,[4] and a 93% reduction with the SP and piperaquine combination in The Gambia[5] where the efficacy of SP is still high.[6]

In the PMC trial (Chapter 6), we recruited children with all-cause severe anaemia as opposed to children with only severe malarial anaemia in the previous trial in Malawi.[1] The reason was that subsequent observational studies showed that in malaria-endemic areas, children with severe anaemia were at an increased risk of post-discharge mortality irrespective of whether they had malaria on admission (Chapter 5). Indeed, this was confirmed in our PMC trial as we did not find a difference in the effect of the PMC between children who had evidence of malaria infection at baseline and those who did not (Chapter 6).

Nevertheless, because of the other similarities in the design and setting of the two studies, we pooled the results in an aggregate data meta-analysis which shows an overall reduction in the primary outcome of 33% by six months (HR=0.67, 0.56-0.78, p<0.001) and 66% during the intervention period (HR=0.34, 0.24-0.43, p<0.001). All cause-readmissions were also significantly reduced by 33% overall (HR=0.67, 0.56-0.78, p<0.001) and 63% during the intervention period (HR=0.37, 0.27-0.47, p<0.001). Similar findings were obtained for the pooled analyses for readmissions due to severe anaemia or severe malaria and clinic visits due to clinical malaria overall and during the intervention period. The pooled reduction in mortality was only significant during the intervention period (HR=0.09, -0.20-0.39, p<0.001) with no effect overall (HR=0.79, 0.28-1.29, p=0.530). None of the outcomes was affected in the post-intervention period when the protective drug levels had waned (Figure 1).

Figure 5: Meta-analysis of the primary outcome (all-cause readmission /death)



HR, hazard ratio; CI, confidence interval; AL, artemether-lumefantrine; DP, dihydroartemisinin-piperaquine.

There have been two previous trials in areas with highly seasonal malaria transmission in West Africa that looked at the impact of malaria chemoprevention when provided to children with severe anaemia during the malaria transmission season. Both were conducted in The Gambia.[7,8] The first was a prospective follow up of children between six months and nine years of age initially admitted and managed for severe malaria anaemia. They were randomised twenty-eight days later to receive either weekly chemoprophylaxis with the pyrimethamine and dapsone combination (Maloprim®) (n=98) or placebo (n=86) for a maximum of twelve weeks during the malaria transmission period. The intervention was found to reduce all-cause readmission (odds ratio

[OR]=0.19, 0.03-0.76, p=0.02) and clinical malaria (OR=0.36, 0.14-0.90, p=0.03), but protection against mortality or recurrence of severe anaemia was not reported.[8] The second study was a placebo-controlled trial providing monthly SP post-discharge to children aged three months to nine years with moderate to severe anaemia (Hb <7g/dL).[7] A total of 1200 children, equally divided between the intervention and placebo arms, were recruited and followed for the rest of the malaria transmission seasons. The duration of follow-up per child dependent on the timing of enrolment in the transmission season with children admitted early in the transmission season being followed for longer. Like our study, there was substantial protection against the recurrence of severe anaemia (PE=78%, -3%-95%, p=0.055) and clinical malaria (PE=53%, 37%-65%, p<0.001).

Although the available evidence is restricted to four trials, it indicates that post-discharge malaria chemoprevention may be beneficial in both areas with intense year-round malaria transmission as seen in our trial (Kenya and Uganda) and Malawi,[1] and in areas with highly seasonal malaria transmission when offered during the malaria transmission season.[7,8] However, these PMC studies in the Gambia were conducted prior to the widespread introduction of SMC. Thus, today, PMC is unlikely to play a role in seasonal malaria transmission areas where SMC strategies are widely implemented as SMC may offer similar protection as PMC in the post-discharge period.

Tolerance and safety of post-discharge malaria chemoprevention

DP was well tolerated in our study (Chapter 6), consistent with reports from previous studies in children. The major safety concern with DP is its dose-related QT prolongation associated with the piperaquine component. Previous studies examining the safety of repeat dosing of DP when used on apparently healthy children did not report any clinical adverse events related to QTcF prolongation.[9] However, no study in malaria-endemic areas has previously reported on the cardiac safety of repeat doses of DP in convalescent children recently discharged from hospital. In our nested cardiac monitoring study involving 33 children from the main trial (Chapter 6), we found a mean (SD) increase of 22ms (14) from baseline to 4-6 hours after the last dose of the first course and similar increases following administration of the second and the third courses. No child had QTc values exceeding 500ms using the Fridericia's correction and one child (3%) had QTc values exceeding 500ms when using Bazett's correction. No clinical cardiac adverse events occurred in any of the 1049 children enrolled. The lack of an increase in QTc prolongation following monthly courses of DP, despite the potential for dose accumulation of piperaquine due to its slow elimination, is consistent with the findings from a previous trial that provided monthly doses to apparently healthy children 6 to 24 months of age, some of whom received 18 monthly courses of

DP. In this study in Uganda, 145 ECGs were taken from 19 of these children showing no difference in the magnitude of QTcF prolongation between the monthly courses; i.e. the mean QTcF prolongation after 18 months was similar to the degree of QTcF prolongation observed with earlier courses.[9] Similar findings were reported in a recent systematic review on the safety of repeat dosing of DP involving a total of 3935 individuals and 18,297 courses of DP (2-18 courses per individual).[10] Although these findings are reassuring, our sub-study only included 33 children and it would be advisable to collect more safety data as part any Phase-IV studies if and when PMC become policy to provide further reassurance of the cardiac safety of DP in this vulnerable target group.

The trial (Chapter 6) included an extended follow-up period until six months post-discharge. The rationale behind this related to the concerns about the interference in the development of anti-malarial immunity in children exposed for prolonged periods of malaria chemoprevention. This could lead to a potential rebound of malaria-related morbidity and even all-cause mortality after the chemoprevention is stopped (Chapter 2), which could cancel out the initial benefits seen during the 3 months intervention period. Some studies have reported a significant rebound in malaria and anaemia in the periods immediately following cessation of the intervention.[11,12] However, most IPTi and IPTc studies did not show a difference in the risk of malaria, anaemia or hospital readmissions between the intervention and placebo arms after the intervention was stopped.[9,13,14] These conclusion from individual studies were consistent with those from a pooled analysis in a systematic review and meta-analysis on the safety and efficacy of IPTi using SP.[15] In our study (Chapter 6), we found a statistically non-significant increase in the risk of mortality during the post-intervention period (HR=2.67, 0.85-8.40) after the protective drug levels had waned approximately 4 weeks after the third course of DP, i.e. by 15 weeks post-discharge; similar findings were obtained in the Malawi trial (HR=1.60, 0.52-4.89),[1] and when the two studies were pooled (HR=1.87, -0.02-3.76). it is unlikely that this reflects a decrease in malarial immunity as there was no corresponding increase in the number of clinical malaria events post-intervention (Chapter 6: HR=0.84, 0.67-1.05; Phiri et al, HR=1.10, 0.89-1.37). It is possible that this reflects artefactual increased mortality due to a frailty effect because the sub-group of the most vulnerable children in the placebo arm is no longer contributing to the post-intervention period because they died before, whereas in the PMC arm mortality is not avoided but delayed until the protective drug levels have waned. By six months the mortality was 26% lower in the PMC arm, but the confidence intervals were wide, and not statistically significant. Overall, it appears that, like the IPTi and IPTc studies, the interruption in the

acquisition of antimalarial immunity, if any, is small, and the benefits of the intervention outweigh the risks. Continued surveillance is inevitable for more conclusive information.

Another concern with the use of malaria chemoprophylaxis is the risk of enhancement of the development of antimalarial drug resistance due to increased drug pressure (Chapter 2). During the PMC trial, we collected blood samples for host and parasite genetic analysis before and after completion of the study. However, these results were not yet available by the time of writing this thesis. Reports from IPTi and SMC trials have mixed findings (Chapter 2) on the effects of widespread use of antimalarials for chemoprevention on the development of drug resistance, hence the need for more evidence and continued surveillance. Therefore, it is important to establish the baseline prevalence of artemisinin[16] and piperaquine[17] markers of resistance before the deployment of PMC with DP. This is important to assess any changes in these molecular markers associated with the implementation of PMC if continuous surveillance is implemented.

Aetiology of post-discharge morbidity and indirect benefits for malaria chemoprevention

In highly malaria-endemic areas, post-discharge malaria has been reported in many studies as a likely cause for post-discharge morbidity and mortality,[18-21] but this has not been adequately quantified, and other diseases and conditions contribute. Studies conducted to determine post-discharge survival of children admitted with severe anaemia have mainly reported on all-cause mortality or all-cause morbidity or recurrence of severe anaemia,[22] however, to the best of our knowledge, no reports on the aetiology of post-discharge mortality among children previously admitted with severe anaemia are currently available from malaria-endemic areas of sub-Saharan Africa. Other aetiologies which co-exist with malaria or whose effects are potentiated by malaria infections may be affected by reductions in the risk of malaria. Several studies have demonstrated that reductions in malaria can have indirect benefits as demonstrated by reductions in all-cause mortality rates following the aggressive deployment of malaria control interventions that were greater than could be expected from an effect of reductions in malaria-related deaths alone.[23,24]

Persisting post-discharge bacteraemia could be a major cause of post-discharge mortality in children recently admitted with severe malaria in malaria-endemic areas of Africa. There is a strong positive correlation between malaria endemicity and invasive non-typhoidal salmonella infections (iNTS) ($p=0.1$; $r=0.70$)[25] and on-going or recent *Plasmodium falciparum* infections are associated with iNTS with in-hospital case-fatality rates approaching 20% irrespective of treatment with suitable antibiotics.[26] A systematic review and meta-analysis investigating the association between

invasive bacterial co-infection and *falciparum malaria* in Africa found that the outcome is even worse for children admitted with severe malarial anaemia and iNTS (24%) co-infection compared to children without malaria (10%).[27] In Chapter 4, we also found a significant positive association between bacteraemia and post-discharge mortality. The clinical presentation of iNTS and malaria overlap and most facilities in malaria-endemic areas in sub-Sahara Africa have limited availability of blood culture services; thus, most of these infections go undiagnosed and untreated. This is reflected in our data collected routinely from a hospital within the KEMRI/CDC HDSS, where the number of children diagnosed with bacteraemia was too low for any analysis to be conducted. It is, therefore, likely that several children were discharged with persistent bacterial infections which worsened after discharge leading to post-discharge mortality. The WHO recommends the use of broad-spectrum antibiotics for all children with suspected severe malaria in moderate to high malaria transmission settings,[28] however the current WHO guidelines for the identification of children with invasive bacterial infections fail to identify about a third of infected children and about 50% of bacterial isolates from these children are not susceptible to the currently recommended antibiotics.[29] The more potent antibiotics like third-generation cephalosporins are not easily available or affordable for routine use in these malaria-endemic areas.[30] Persisting post-discharge bacteraemia, including iNTS and its contribution to the burden, needs to be quantified further.

Severe malaria vs non-malarial anaemia and the post-discharge burden

In Chapter 3 and 4, we found that the risk of six months post-discharge mortality was significantly higher among children admitted with non-malaria severe anaemia compared with those with severe malarial anaemia. One potential reason for this difference may be misclassification of uncomplicated malaria as severe malaria, which is very common in Africa, where as many as one-third of admissions for 'severe malaria' do not fulfil WHO's criteria for severe malaria.[31] Another potential explanation is the challenge in diagnosing these non-malaria causes of severe anaemia relative to diagnosing malaria-associated anaemia, resulting in inadequate in-hospital management and children being discharged with persisting underlying conditions. In many lower-level facilities in malaria-endemic areas, anaemia and malaria are diagnosed clinically, and in-hospital treatment for severe anaemia, even non-malarial severe anaemia constitutes of blood transfusion and parenteral antimalarial treatment, which is instituted presumptively following the IMCI guidelines,[32,33] without much further investigation to the cause of anaemia. Coupled with delays and poor health-seeking behaviour[34] children with non-malarial severe anaemia are thus more likely to die in the post-

discharge period without a diagnosis and proper treatment of the underlying condition than children where malaria was the primary cause of the severe anaemia.

The WHO recommends the rational use of blood for transfusion to reduce unnecessary use and transfusion-transmitted infections. The adherence to these guidelines is poor leading to high rates of in-hospital mortality among severely anaemic children in sub-Saharan Africa,[35] especially if they had severe malaria anaemia and respiratory distress.[36] Due to frequent shortage and/or delay in transfusion,[37] (also observed during the PMC trial) it is possible that some children are discharged with no or inadequate transfusion. These children are likely to be readmitted because of recurrence of severe anaemia or are exposed to a higher risk of post-discharge mortality as has been observed in previous studies,[38,39] especially if they come from households with poor health-seeking behaviour.

Vitamin A deficiency is a neglected cause of severe anaemia in sub-Saharan Africa, yet it is a widespread micronutrient deficiency in Africa.[40] It has also been shown to potentiate the susceptibility of children to malaria infection and bacteraemia,[40,41] both of which are possibly associated with post-discharge mortality. Vitamin A deficiency could be associated with severe acute malnutrition in children in malaria-endemic areas, a condition we found (Chapter 3 and 4) to worsen the post-discharge mortality outcomes in children with severe anaemia.

Red cell production failure (RCPF) has been shown to be a common pathway in the pathogenesis of severe anaemia in sub-Saharan Africa and can be triggered by many aetiological factors discussed above. Other conditions which are known to cause RCPF are viral infections including HIV infections which is prevalent in malaria-endemic areas and a possible cause of post-discharge mortality and morbidity. HIV positive status has been shown to be positively associated with post-discharge mortality as shown by our study (Chapter 3) and others.[42,43] However, like the Malawi study, we did not observe a difference in effect based on HIV status. Data may be insufficient to make any conclusions in each of the two studies, but since the HIV positive children were on co-trimoxazole prophylaxis, PMC may provide less additional antimalarial protection than in HIV uninfected children since co-trimoxazole is an effective anti-malarial drug.[44]

Risk factors of post-discharge mortality and morbidity

In addition to determining the aetiology of post-discharge mortality, identification of clinical or demographic predictors associated with post-discharge mortality present on admission or discharge could offer a cost-effective option for identifying children who are at greatest risk of

post-discharge mortality and may benefit from targeted management. Most countries in malaria-endemic sub-Saharan Africa are resource-constrained and therefore following all children after discharge may not be feasible in the routine healthcare system. Although this thesis did not include a detailed study of the clinical risk factors and other predictors of post-discharge re-admission or mortality, in Chapter 3, we provided the risk factors associated with post-discharge mortality as identified on admission, but our data was limited and did not include the clinical parameters on admission to hospital. To date, we are aware of two studies; one in Uganda[45] and a more recent one in Mozambique, which developed risk scoring algorithms for predicting post-discharge mortality using simple variables collected on admission. These models are intended to provide more targeted post-discharge care. The primary models in the two studies identified about 80% of children who are at risk of post-discharge mortality.[42,45] According to these models, poor anthropometric measurements and younger age are predictive of post-discharge mortality, characteristics which were also identified in our study to be associated with post-discharge mortality (Chapter 3). Other risk factors commonly identified and included in the primary prediction models in the two studies were HIV positive status, hypoxia and abnormal conscious levels. These models are likely to provide a more objective and reliable means of identifying children at risk of post-discharge mortality, but the low specificity and positive predictive values reported in the models in the two studies are likely to compromise the effectiveness of post-discharge management.

Implications for future research

This thesis raises several important gaps in the overall management of severe anaemia in malaria-endemic areas of Africa, which is currently focussed on in-hospital care. Post-discharge mortality is a major burden in this setting in children previously admitted with severe anaemia. Post-discharge malaria infection has been shown to be an important underlying condition leading either directly or in-directly to morbidity and mortality. This study did not assess the aetiology of post-discharge morbidity and further studies to determine this, and the magnitude of malaria versus non-malaria conditions leading to morbidity or mortality post-discharge are warranted. There is a need to assess the contribution of poor adherence to the current standard guidelines for the in-patient management of severe anaemia in-hospital and their link to post-discharge mortality. A recent study in Uganda showed that strict adherence to clinical guidelines was associated with a significant reduction of in-hospital deaths associated with severe anaemia.[46] As discussed above, improvement of the discharge process through the identification of children most at risk may

reduce the burden of post-discharge mortality through improved and focused management restricted to the highest risk population. More data, however, is needed to develop more robust prediction models by incorporating data from varied settings in malaria-endemic sub-Sahara Africa, possibly through a systematic review and meta-analysis of relevant studies in this setting.

Based on the possible breath of aetiologies for post-discharge mortality and morbidity in children with severe anaemia in malaria-endemic areas, additional post-discharge interventions to cover a broader range of aetiologies may improve the efficacy of PMC on all-cause post-discharge mortality and morbidity. As discussed above, aggressive deployment of malaria control interventions has been linked to substantial reductions in all-cause mortality at levels higher than can be attributed to reductions in malaria alone. The contribution of non-malaria causes of post-discharge morbidity and mortality has not been fully quantified, but malaria remains a common underlying factor in this setting.[18-20] Several factors have been postulated to increase the hosts' susceptibility to other infections following clinical malaria, including prolonged neutrophil impairment and immunological dysregulation.[47,48] Inflammatory processes from bacterial and other infections lead to disruption of surface receptors on endothelial walls which forms the pathological basis for many infections including sepsis and *plasmodium falciparum* malaria.[49,50] It has been shown that endothelial inflammation persists for more than a months following *Plasmodium falciparum* infection, even after the parasites have been eliminated,[51] a situation which may persist for longer in malaria-endemic areas because of repeat malaria infection and other infectious agents prevalent in this settings. This would be consistent with the finding that most post-discharge deaths occur in the immediate post-discharge period; this was >50% in the first one month in other studies,[42,45,52] although it was only about 60% in the first three months in our study.

The addition of a broad-spectrum antibiotic to PMC may have more beneficial effects than PMC alone. For example, the association of iNTS and malaria and its associated high in-hospital mortality in children with severe anaemia has been confirmed in several studies [26,27] and is possibly associated with high post-discharge mortality if not adequately managed. Azithromycin is a potent broad-spectrum antibiotic, also with well documented, although modest, antimalaria properties.[53,54] It has been widely used for mass drug administration for the control of Trachoma in West Africa with over 100 million doses being distributed annually with no serious safety concerns.[55] An observational study during a mass drug administration exercise in Ethiopia for the control of Trachoma using a single dose of azithromycin found a significant reduction in all-cause mortality among children one to five years of age who received the intervention 26 months earlier

(OR=0.35, 0.17-0.74)[56] and similar findings were reported in the same setting among children 1 to 9-year-old in a cluster-randomised trial.[57] These findings were further confirmed in a large cluster-randomised trial (Mortality Reduction After Oral Azithromycin [MORDOR]) which involved more than 190,000 children in three sub-Saharan African countries. Azithromycin was distributed half-yearly for two years, and a significant reduction in all-cause mortality was reported among the communities that received the intervention with the greatest reduction being in children between 1 to 5 years of age (RR=0.75, 0.63-0,89, p<0.001).[58] However, the addition of azithromycin to monthly SP and amodiaquine combination for SMC in Mali and Burkina Faso did not result in a reduction in mortality or hospital readmissions.[13] The authors of this SMC trial attributed the no-effect findings to several reasons including that the mortality in MORDOR trial may have been due to the antimalaria effects of azithromycin which were cancelled out in their trial since all children received SP and amodiaquine combination which is a more efficacious antimalarial than azithromycin. Furthermore, the antimicrobial effects of SP may have reduced the benefits of azithromycin and lastly, the coverage of pneumococcal conjugate vaccine was high in their trial sites. Nevertheless, the incidence of the gastrointestinal, upper respiratory tract and other febrile infections were significantly reduced in the azithromycin arm. Therefore, because of the high incidence of iNTS infections in children with severe anaemia in malaria-endemic areas, the addition of azithromycin to PMC may have greater beneficial effects than those observed in SMC participants who are generally healthy individuals at risk of malaria. Studies to determine further benefits of adding azithromycin to PMC are warranted, however because of safety concerns due to the concomitant administration of DP and azithromycin, both of which are known to cause QT prolongation, an alternative broad-spectrum antibiotic or alternative antimalarials may be considered, or studies to determine the safety of DP and azithromycin co-administration may be needed.

DP has been evaluated in several trials looking for alternative drugs for IPTp because of the widespread resistance of *plasmodium falciparum* to SP.[6] It has been shown to have better anti-malaria prophylactic effects than SP. However, despite the high resistance, SP has been associated with better or similar pregnancy outcomes compared to DP possibly because of the additional non-malaria properties of SP; e.g. through its antibiotic effects or potential anti-inflammatory effects as were demonstrated for cotrimoxazole. An individual participant data meta-analysis of three recently completed trials comparing the efficacy of IPTp with DP and SP shows that SP has a potent non-malaria effect on birth weight (ter Kuile et al, personal communication), which explains the

higher mean birth weight in the SP arms relative to IPT with DP observed in these trials. The combination of DP plus SP would possibly result in better outcomes among severely anaemic children because of the antibiotic/anti-inflammatory effects of SP in addition to the significant outcome of a reduction in post-discharge mortality and morbidity due to the anti-malarial effects of DP seen in our trial in this setting.

Another possible regimen is an azithromycin-chloroquine combination, both of which have anti-malarial and anti-inflammatory properties with well-established safety profiles. Co-administration of azithromycin and chloroquine has been shown to have synergistic effects against *Plasmodium falciparum* infections. Azithromycin-1000mg and chloroquine 600mg base combinations administered once daily for three days has been shown to achieve 28 days malaria-free periods of >98% in adults in two clinical trials in sub-Saharan Africa. Chloroquine monotherapy was previously evaluated in a clinical trial with the hypothesis that the anti-inflammatory effects of chloroquine against *Plasmodium falciparum* induced prolonged inflammation may potentiate erythropoietic recovery, but the findings were indifferent with the placebo arm, possibly because of the low dose of chloroquine used and recruitment of participants during a low malaria transmission season.[59] Because of the anti-malarial and antibiotic effects of chloroquine and azithromycin, use of a treatment dose of chloroquine in combination with azithromycin merits an evaluation among children admitted with severe anaemia in malaria-endemic areas to reduce post-discharge mortality and morbidity.

Another possible intervention worth exploring in the addition of vitamin A into the PMC regiment (or Vitamin A bolus on discharge) to improve on Vitamin A associated causes of severe anaemia. As discussed above, Vitamin A deficiency has been shown to be a major factor leading to severe anaemia in sub-Sahara Africa[40] and Vitamin A supplementation has been shown to reduces the incidence of malaria infection and parasite densities.[60] Another randomised controlled trial in Tanzania also found that the addition of Vitamin A and C to monthly SP for management of children with mild malarial anaemia was associated with improvements in the mean serum transferrin receptor levels compared to children who received SP alone.[61]

PMC Delivery mechanism

The studies described in this thesis were part of the activities of a PMC Consortium. This included a delivery mechanism trial in Malawi. This cluster-randomised controlled trial in Malawi compared community-based delivery mechanisms of PMC (drugs for all PMC courses were given to caretakers

at the time of discharge) versus facility-based approaches (drugs collected monthly by caretakers at the facility) with and without SMS reminders. It was found that community-based delivery achieved higher adherence (Nkosi-Gondwe, unpublished). A nested study among the caregivers showed that PMC was highly acceptable and home-based delivery together with SMS reminders was preferred to the health-facility collection of drugs or reminders from healthcare workers due to convenience and economic reasons.[62] No conclusions could be made regarding the addition of SMS reminders because very few were delivered to the intended participants due to low mobile phone usage and coverage in Malawi. SMS reminders have been deployed in the management of various health issues in Africa with mixed results,[63] therefore further studies to evaluate the addition of SMS reminders in the delivery of PMC are warranted.

The WHO recommends the utilisation of existing community platforms for the delivery of SMC.[64] Randomised-controlled trials have shown that the delivery of SMC by community health workers (CHWs) can achieve high coverage and is cost-effective.[65] However, utilisation of CHWs in existing routine programmes has been shown to achieve less coverage. In a study in Mali, 84% of eligible children received the first course of SMC and only 54% completed the four recommended courses.[66] In Ghana, there was no difference in the completion rates of all the three doses of IPTc when it was delivered at the community by CHWs or at the health facilities (91.6% and 91.7%).[67] In Malawi, less than half of the CHWs made the required reminder home visits mainly due to high workload, inadequate training, low community acceptance and other organizational challenges.[68] PMC may be another addition to the workload, therefore, further evaluation of delivery options for PMC are warranted.

Policy implications

The current standard practice for the post-discharge management of severe anaemia in malaria-endemic areas entails the provision of artemether-lumefantrine at discharge if malaria was suspected and a short course of iron and folate (Chapter 5). PMC offers a potential strategy to reduce the high post-discharge morbidity and mortality observed in this population. Similar strategies which have been successfully deployed for the control of malaria including IPTp, IPTi and SMC (Chapter 2). Therefore, PMC is a potential new strategy to reduced childhood mortality and morbidity if found to be acceptable and cost-effective and if a sound delivery mechanism for the delivery of the intervention can be found.

Furthermore, the epidemiologic and geographical areas where PMC would be a cost-effective strategy needs to be determined. This involves identification of the optimal target population of children, the proportion that access hospital care and get adequate management, and their geographical location in the malaria-endemic setting. A similar strategy to identify these targets group and geographical areas were used to identify the appropriate areas for SMC deployment.[69] To support this process we conducted the systematic review and meta-analysis of studies reporting the burden of severe anaemia and the post-discharge risk of morbidity and mortality in malaria-endemic areas of Africa (Chapter 4). A next step is to combine the results of our trial (Chapter 6) with the three previous efficacy trials in Malawi[1] and the Gambia[7,8] using a formal individual-level participant data meta-analysis to obtain a summary effect measure overall and by transmission strata (perennial vs highly seasonal) This information would then be used in a spatially-stratified mathematical model to simulate the impact of the intervention on morbidity in the presence of the existing malaria control interventions (e.g. ITN, IRS, mass drug administration, malaria vaccine etc.) at various levels of deployment. These activities are currently being conducted by other groups as part of the PMC consortium.

In order to facilitate the adoption of PMC as a management strategy for severe anaemia, the consortium included a policy taskforce as one of its major objectives. Local senior paediatricians and health managers were involved in the protocol development. In addition to ethical and regulatory approvals, the study protocol was reviewed and approved by the Ministry of Health and the regional referral hospitals in both countries. Some senior hospital staff also supported the training of study personnel and facilitated other aspects of the study, such as laboratory tests and sample storage before shipment to the research laboratories. We conducted sensitization meetings in all study hospitals and to regional/County health management teams before and after the study was concluded. During these meetings, the study rationale was shared in detail; we realised that, outside of a research setting, the hospital staff have limited awareness of the burden of the post-discharge burden of severe anaemia in this malaria-endemic setting. We stressed that this study is designed to complement the efforts of reducing under-five mortality, a struggle which has mainly concentrated on reducing in-hospital mortality in the past decade. The study findings will be presented to the Kenya National Malaria Control Programme in the second quarter of 2020. The Ugandan National Malaria Control Programme is already considering including PMC in their Malaria strategy because of the impressive study findings. The study findings have been presented to the WHO and further discussions are ongoing.

Limitations

Most of the limitations identified in this thesis have been discussed in the respective chapters, and further limitations will be highlighted in this section.

In Chapter 3, we used data from one hospital within the KEMRI/CDC HDSS, and this being the major referral hospital there is a possibility that only the most severe cases were considered in this retrospective analysis. There are other lower-level hospitals within the HDSS area that provide in-patient care, but no paediatric in-patient surveillance was on-going in these other hospitals. It is thus possible that mortality rates are underestimated in our studies if they were referred to these other hospitals. By contrast, it is also possible that the overall mortality rates are overestimated if the most severe cases were referred to the study hospital from these lower-level facilities. A retrospective cohort analysis of HDSS data in Mozambique found a significant positive association between transfer from other facilities and post-discharge mortality.[42]

In Chapter 3 and 4, we excluded children with sickle cell anaemia and other haemoglobinopathies, trauma, surgical conditions and malignancies in estimating the risks of post-discharge mortality and morbidity. A recent post-discharge mortality study in Tanzania among the general paediatric population reported that chronic conditions such as cancer, cardiovascular diseases and sickle cell anaemia were significantly more associated with post-discharge mortality than other conditions.[52] It was also not clear if these may have been included under `undefined conditions` in the studies included in Chapter 4 because they were not expressly mentioned. The burden of post-discharge mortality may, therefore, be underestimated. Further, the determination of the post-discharge risks of mortality associated with chronic conditions in malaria-endemic areas is worth exploring because of their associations with severe anaemia. In Chapter 4, we did not find sufficient data on post-discharge morbidity and mortality for children admitted with diarrhoea, yet it is a common cause of paediatric admission in resource-poor countries.[70] The limited focus on post-discharge mortality associated with diarrhoea in sub-Saharan Africa was highlighted in a recent publication where they noted that since 2013, 17 more studies on diarrhoea have been published with none reporting on post-discharge mortality.[71] This paucity of data on follow-up studies on paediatric diarrhoea precluded any comparative analysis of post-discharge mortality risk of children admitted with diarrhoea and anaemia or other conditions in our systematic review. Similar challenges were faced with data on bacteraemia, possibly because of inadequate diagnostic and culture facilities in most facilities in this setting.

The search for studies included in the systematic review and meta-analysis in Chapter 4 was closed in October 2018, and since then, more studies have been published in sub-Sahara Africa on post-discharge mortality and morbidity and thus are not included in this thesis.[42,71,72] The new evidence which has come up will be included in future updates of our systematic review and meta-analysis.

A limitation in the PMC trial is the relatively short duration of the intervention period. We observed a 70% reduction in the incidence of the primary outcome during the intervention period and the effect was absent in the subsequent post-intervention period. A significant number of events occurred between 15 and 26 weeks, 47% (126/184) of all events post-randomisation; 34% (107/316) in the placebo arm and 68% (233/500) in the PMC arm. It is thus likely that more events would have been prevented had the intervention period been longer. Studies to determine the optimum duration for the PMC intervention are warranted.

Conclusions

This study shows that children admitted with acute conditions in malaria-endemic areas in sub-Saharan Africa are at a high risk of post-discharge mortality and morbidity in the first few months during the post-discharge period. Admission with all-cause severe anaemia in this setting is associated with a doubling of the risk of post-discharge mortality compared to other acute conditions. Coexistence of severe acute malnutrition further worsens this risk. Malaria chemoprevention with three monthly courses of DP is a promising tool for post-discharge management of children recently admitted with severe anaemia in malaria-endemic areas. Further studies of effective delivery mechanisms are now warranted.

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